STUDY OF VOLATILE COMPOUND FORMATION IN OXIDIZED LIPIDS AND VOLATILE COMPOUND RETENTION IN PROCESSED ORANGE JUICE

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Jeffrey Michael Boff, B.S.

* * * * *

The Ohio State University
2003

Dissertation Committee:
Professor David B. Min, Advisor
Professor Hua Wang
Professor Polly Courtney

Approved by

_____

Advisor
Food Science and Nutrition

ABSTRACT

The production of volatile off-flavor compounds by lipid autoxidation is one of the most widely recognized quality concerns in foods. Within the past 30 years, increased attention has been given to singlet oxygen oxidation. Photosensitizers such as chlorophyll in foods can produce singlet oxygen, which accelerate oxidation and produce volatile off-flavor compounds. The effect of chlorophyll concentration on the formation of volatile compounds in soybean oil, peanut oil and lard has not been thoroughly studied.

Soybean oil, peanut oil and lard containing 0 to 10 ppm chlorophyll was stored under light for 7 to 10 days at 4, 20 and/or 40 ^{o}C. Headspace volatile compounds were collected by solid phase microextraction and analyzed by gas chromatography and mass spectroscopy. Volatile compounds in soybean oil, peanut oil and lard with or without added chlorophyll were qualitatively similar after 7 to 10 days of light exposure. The presence of 5 and 10 ppm chlorophyll in soybean oil, peanut oil and lard significantly increased the production of approximately half of the volatile compounds in the soybean oil and lard after only 1 to 2 days of light exposure. The amounts of pentane, pentanal, 1-pentenol, 2-pentenal, hexane, 1-hexene, 2-hexanal, heptanal, 2-heptenal, 2-pentyl furan, 2,4,-heptadienal, 2,4-nonadienal, 1-octen-3-ol and 2-octenal in the lipids were significantly affected by chlorophyll concentration and are likely primary products of

singlet oxygen oxidation. The presence of chlorophyll was the most significant factor to the amount of chlorophyll sensitive compounds produced in soybean oil during the first 2 days of light exposure; however, temperature was the most significant factor after 2 days of light exposure. Fatty acid composition was a significant factor in the amounts of volatiles produced in lipids containing chlorophyll indicating that autoxidation reactions are an important contributor to the production of volatile compounds imitated by photosensitized reactions. Relative to the triplet oxygen product hexanal, the singlet oxygen product 2-heptenal increased in quantity rapidly in soybean oil or peanut oil containing 5 ppm or 10 ppm chlorophyll when exposed to light even at 4 °C. The presence of chlorophyll was the most significant factor to the amount of volatile compounds during the first 2 days of light exposure; however, temperature was the most significant factor after 2 days of light exposure.

Orange juice is the most predominant fruit juice in the U.S. market today. Commercial orange juice is thermally processed to inactivate pectinmethylesterase (PME) and spoilage organisms. However, thermal processing can be detrimental to the organoleptic and nutritional qualities of the juice. Previous research showed that the addition of CO_2 to orange juice prior to high pressure processing (HPP) resulted in decreased volatiles after processing relative to the HPP alone. HPP in combination with added $CO_{2(g)}$ was examined for efficacy in inactivating Valencia orange juice PME. Post-process chemical changes in ascorbic acid and headspace volatiles were evaluated in HPP treated samples, with and without added $CO_{2(g)}$. The effects of high-pressure processing (HPP), CO_2-assisted high-pressure processing (HPP+CO_2), and thermal

processing on the chemical and physical properties of single-strength Valencia orange juice were evaluated over 4 months of storage at 4 and 30 ºC. Pressure magnitude, temperature, and dwell time were significant factors ($p < 0.001$) in the inactivation of PME. Gas concentration increased the rate of PME inactivation ($p < 0.001$) across all of levels of pressure, temperature, and time. Low-molecular-weight compounds were shown to be lost more readily than higher-molecular-weight compounds as the amount of added CO_2 increased. The HPP+CO_2 juice had the greatest cloud stability and highest ascorbic acid retention during storage at 4 and 30 ºC. $CO_{2(g)}$ addition enhances PME inactivation beyond that achieved by high pressure alone. Pressure treatments resulted in insignificant volatile losses and minor ascorbic acid reduction. Volatile compound losses were lowest in the HPP juice and lower in the HPP+CO_2 juice compared to the thermally processed juice ($p<0.05$). HPP+CO_2 produced a cloud-stable orange juice with more ascorbic acid and flavor volatiles than thermally processed juice ($p<0.05$).

Dedicated to my wife and parents

ACKNOWLEDGMENTS

I must thank Dr. David Min for all of the support, opportunities and patient guidance that he provided during my study and Dr. Tom Shellhammer who I worked with extensively for the completion of the high pressure processing project. You have been great teachers, advisors and mentors who have helped to shape my education and my life. I also thank my committee members, Drs. Zhang and Courtney for their advice and guidance. Special thanks to Tommy Truong with whom I conducted the high pressure processing research. My colleagues and friends have played an integral part in the successful completion of my graduate career. They have provided support and advice throughout. Kyle Kent, Jae Hwan Lee, Amanda Callison, Steven Howard, Charles Streker and Don Steenson. Thank you. I have been fortunate to be a part of the OSU Department of Food Science for eight years. Numerous members of the department have made significant contributions to my education. Special thanks to my undergraduate advisor Dr. Grady Chism, my honors project advisor Dr. Ahmed Yousef and a non-official advisor Dr. Mike Mangino. Thank you for your time and guidance.

Most importantly, I sincerely thank my wife, parents, grandparents, brothers and sisters for their contributions. I am very fortunate to have had such enthusiastic and liberal support for my pursuits in science and education throughout my life.

VITA

June 14, 1977 ---------- Born – Defiance, Ohio

1995-1999 ---------- B.S. Food Science and Technology,
The Ohio State University

2000 – 2003 ---------- Graduate Fellow, The Ohio State University

PUBLICATIONS

1. **Boff, JM** and Min DB. 2003. Making Sense of Taste. Food Quality Magazine. 10:36-39.

2. **Boff, JM**, Truong, TT, Min DB and Shellhammer TH. 2003. Effect of Thermal Processing and Carbon Dioxide Assisted High Pressure Processing on Pectinmethylesterase and Chemical Changes in Orange Juice. Journal of Food Science. (Accepted, Volume 68)

3. Truong, TT, **Boff, JM**, Min, DB and Shellhammer TH. 2002. Effects of carbon dioxide in high pressure processing on pectinmethylesterase in single strength orange juice. Journal of Food Science. 67:3058-3062.

4. Min, DB and **Boff, JM**. 2002. Crude Fat Analysis. In: Nielsen, SS, editor. Food Analysis, 3rd Edition, Ch. 8. Aspen Publishers, Inc., Gaithersburg, MD. (In Press)

5. Min, DB and **Boff, JM**. 2002. Chemistry and reaction of singlet oxygen in foods. Comprehensive Reviews in Food Science and Food Safety. 1:1-15.

6. Min, DB and **Boff, JM**. 2001. Lipid oxidation of edible oil. In: Akoh, CC and Min, DB, editors. Food Lipids: Chemistry, Nutrition and Biotechnology, pp 335-363. Marcel Dekker, Inc., New York.

PUBLISHED ABSTRACTS

1. **Boff, JM**, Shellhammer TH and Min DB. 2002. Ascorbic Acid and Volatile Chemical Retention of High Pressure and Thermally Processed Valencia Orange Juice. IFT Annual Meeting.

2. **Boff, JM**, Shellhammer TH and Min DB. 2001. The Impact of CO_2 Assisted High Pressure Processing on Fresh Orange Juice Flavor and Ascorbic Acid Content. IFT Annual meeting.

3. **Boff, JM**, Lee JH, Steenson, DF and Min DB. 2001. Solid Phase Microextraction Analysis of Volatile Compounds in Edible Oils. AOCS Annual meeting.

4. **Boff, JM**, Truong, TT, Shellhammer TH and Min DB. 2001. Effect of Thermal Processing and Carbon Dioxide Assisted High Pressure Processing on Volatile Compound and Ascorbic Acid Retention during storage. Center for Advanced Processing and Packaging Studies Spring meeting.

FIELD OF STUDY

Major Field: Food Science and Nutition

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

This dissertation covers two areas of research: (1) analysis of the production of volatile compounds in lipids due to photoxidation and photosensitized oxidation and (2) the analysis of volatile compound retention and ascorbic acid retention in orange juice processed by CO_2 assisted high pressure processing. Both topics of research involve the analysis of volatile compounds in foods. In lipids, development of volatile compounds is detrimental to the quality of the lipid. In orange juice, the loss of volatiles is detrimental to the quality. Analysis of volatiles in both products was performed using solid phase microextraction, a relatively new technique for volatile analysis.

The main focus of this dissertation is the research involving the photosensitized oxidation and photoxidation of lipids in the production of volatile compounds. The volatile compounds produced in oxidation are important contributors to off-flavors in lipids. Chapter 1 of the literature review on flavor chemistry will appear in the 2003 May/June issue of Food Quality Magazine. Several of the topics that are briefly discussed in Chapter1 are discussed in further detail in other chapters; however, it provides some background information on the production and analysis of volatile flavor compounds in foods and serves as a good introduction to the dissertation research. Chapter 2.1, *Singlet Oxygen Oxidation of Foods*, was written for the online IFT journal: Critical Reviews in Food Science and Food Safety. This article thoroughly describes the

chemistry and reactions of singlet and triplet oxygen and the photosensitized formation of singlet oxygen in foods. Chapters 3-5 present the research conducted on the effect of chlorophyll, light exposure and storage temperature to the production of volatile chemicals in soybean oil, peanut oil and lard, respectively.

Chapters 6 and 7 detail the effect of carbon dioxide assisted high pressure processing on the ascorbic acid and volatile compound retention of orange juice. Both are published in the Journal of Food Science. My contribution to the orange juice research was to perform the volatile compound and ascorbic acid analysis. I also assisted with the preparation of samples and storage design.

CHAPTER 1

FLAVOR CHEMISTRY

Flavor can be difficult to define by words and difficult to characterize by chemical analysis. Product developers may spend many hours and dollars trying to perfect it in the lab and in again in full-scale production. We are concerned about its loss in many products, but its development in many others. Desirable flavors may develop with heat, as is the case for meats and baked goods. Desirable flavors may be destroyed or undesirably altered with heat, as is the case with many fruits and vegetables. Obtaining and maintaining the right flavor may be one of the most difficult hurdles to overcome in formulation and production, or it may be a fairly simple modification or addition to an existing product.

Flavor can often present a difficult challenge, but there is no denying that it plays a critical role in product development and processing considerations for many products. After all, how long can a product remain profitable if a consumer doesn't like how it tastes? The importance of flavor does not go unnoticed in research or industry application. Approximately 15% of the over 900 displays at the 2001 IFT Food Expo were companies that supplied flavors, flavor ingredients, flavor bases or flavor enhancers. Industry and academic researchers are continually studying the chemical and physical

processes that produce or reduce flavor compounds in foods. Many nonthermal processing techniques, such as pulsed electric field processing and high pressure processing, are being evaluated not only for their ability to reduce microbial hazards, but also for their effect on flavor.

What we perceive as "flavor" is a complex interaction of numerous chemicals. It is widely recognized that taste and smell are essential to the perception of flavor. Other factors such as appearance, color, temperature and texture can impact the perception of flavor. The variety of flavors that can result from the interaction of chemicals with the taste receptors on the tongue is limited to combinations of sweet, sour, salty and bitter. Most of the flavor that we perceive is due to the numerous combinations of different volatile compounds at different concentrations, resulting in the "aroma" of the product.

Volatile Compounds

Due to the importance of volatile aroma compounds to flavor, much of the analytical research that has been conducted in flavor chemistry involves the identification and quantification of volatile compounds in foods. This can be a very challenging task given the variability and sensitivity of flavor volatiles. The threshold for perception of volatile compounds, i.e. the concentrations at which their presence is detectable by our senses, may be from several parts per trillion to several thousand parts per million (Table 1). Instrumental detection of compounds with such small concentrations can be extremely difficult, particularly in a food system containing several tens or several hundred volatile compounds.

The chemistry involved in the production of flavor volatiles in foods is dependent on the composition of the food and the environment to which it is subjected. Oxidation of the essential fatty acids linoleic acid and linolenic acid can produce volatile compounds that contribute to the “melon-like” or “cucumber odor” of cucumbers, the “fresh” or “green” odor of tomatoes, the rancid flavors in vegetable oils or the desirable flavors of fried foods; very different aromas from the same substrate. Some of the more complicated flavor profiles are a result of thermally induced reactions of reducing sugars and amino acids resulting in the production of roasted or savory flavors. Roasted coffee, processed meat and roasted peanuts are known to contain 300 to 600 flavor compounds. For comparison, many fruits and vegetables may contain less than 100 to 200 flavor volatiles (Ensor).

The Flavor Chemistry of Lipids

Oxidation of the fatty acids composing fats and oils can produce reactive free radicals, which propagate further fatty acid oxidation, ultimately resulting in the production of undesirable volatile compounds that contribute off-flavors. Several factors such as elevated temperature and light exposure can increase the rate of oxidation reactions of fatty acids. The presence of photosensitizers, such as chlorophyll or riboflavin, in foods can greatly increase the rate of lipid oxidation. Lipid oxidation is generally regarded as an undesirable reaction because it commonly results in the production of volatile compounds that contribute rancidity and off-flavors to foods. However, reactions involving lipids also contribute positive flavors to some vegetables, mushrooms and fried foods.

Unsaturated fatty acids are more susceptible to the initiation of oxidation reactions than saturated fatty acids. The essential fatty acids, linoleic acid and linolenic acid, are unsaturated fatty acids found in many foods, particularly in oils derived from plants. The types and amounts of volatiles formed by oxidation reactions is dependent on the types and amounts of fatty acids present in the system. Further secondary reactions can produce various other volatile compounds. Many of the volatile compounds produced by autoxidation of fatty acids can contribute a "rancid" flavor.

Frying

Some flavors derived from lipid oxidation are desirable. Production of the pleasant flavors of fried foods is critically dependent on the oxidation reactions of oils. The reaction mechanisms that produce undesirable flavors in some products and desirable flavors in fried foods are essentially the same, as are the types of chemicals formed. The critical difference is in the relative amounts of the chemicals formed. In the high temperature and low oxygen environment of frying, greater amounts of low-volatile compounds are produced and lower amounts of high-volatile compounds, secondary products, and hydroperoxides are produced. This, in combination with the production of thermally induced flavors, results in the aroma of fried foods.

Enzymatic effects on flavor

Enzymatic processes are important to the oxidation of fatty acids and production of flavor volatiles in fruits and vegetables. The resultant volatiles may contribute both positive and negative flavors. The lipoxygenase pathway is a series of reactions

involving enzymatic alterations to unsaturated fatty acids. The enzymes associated with this pathway can catalyze both the formation of hydroperoxides from linoleic and linolenic acids and the subsequent degradation of those hydroperoxides into volatile compounds. For example, the activity of hydroperoxide lyase can produce the volatile compounds that are responsible for the beany flavor of beans. A different hydroperoxide lyase in tomatoes produces several 6-carbon compounds that are largely responsible for the flavor and aroma of tomatoes. A 9-carbon compound, *trans*-2-*cis*-6-nonadienal, in cucumbers is the major contributor to the flavor of cucumbers. This compound is also the result of the lipoxygenase pathway.

Carbohydrates and Proteins

Thermally induced or browning flavors such as roasted, nutty, burnt, caramel, toasted or meaty flavors are derived primarily from compounds produced by Maillard browning reactions. Amino acids and reducing sugars react to produce a variety of heterocyclic compounds that provide these browning flavors. The identity and quantities of flavor compounds produced from Maillard browning reactions are dependent on the reactants and influenced by temperature, water activity and time. Therefore, not only the food, but also the method of preparation significantly affects the resulting flavors. Selecting the appropriate substrates, reaction environment and heating conditions can result in the production of specific desirable compounds. Several proteins or protein-rich ingredients may be added to food for the purpose of providing meat-like flavors or enhancing flavors. Hydrolyzed vegetable protein, cysteine, glutathione, methionine and monosodium glutamate may be used for such a purpose.

Measuring Flavor

As understanding of the structure, interactions and origins of flavoring compounds have developed over time, so too has the integration of this knowledge into the development and production of foods. Instrumental analysis of flavor is a relatively recent development largely due to technological advancements in equipment and methods designed for the isolation, detection and identification of volatile compounds. Since the mid 1960s, the number of identified flavor constituents in foods has increased drastically.

Gas chromatography (GC) is the primary tool for flavor analysis. A GC system consists of three primary parts, the injection port, the column and the detector. A liquid or gaseous sample is injected into the injection port of the GC, volatilized by the high temperatures of the injection port, and carried through the column to the detector. The compounds in the sample have different affinities for the column and therefore will be retained by the column at different rates. This allows the mixture of compounds in the sample to be separated. Once separated, both the amount and identity of the compound in the sample can be determined. Each compound will appear as a peak with a specific retention time. The area of the peak is proportional to the concentration of the compound in the sample. Analysis by GC can provide a vast amount of information about the volatile composition of a product, since it can be used to track the losses or increases of specific or total volatiles in a product. In soybean oil, less flavor volatiles is better. Over time, soybean oil is oxidized, resulting in the production of numerous volatile compounds (Figure 1).

The simplest form of collecting volatiles for analyses is the removal of a sample of air from the headspace of a sample contained within an enclosed package. This method can be quite limited because of the low concentrations of volatiles that may be present in the headspace. Numerous methods have been developed to concentrate volatiles from foods prior to instrumental analysis, which have relative advantages and disadvantages. Most methods involve the use of solvents for extraction and distillation for concentration. The procedures involved in the collection of food volatiles are often time consuming and may require very specific equipment. The elaborate nature of some of these collection procedures may provide results that are difficult to reproduce.

Solid Phase Microextraction

A fairly new method for the collection and concentration of volatiles from foods is solid phase microextraction (SPME). SPME offers a very simple and rapid method for collecting and concentrating volatiles compared to other concentration methods involving solvent extraction or distillation.

A fiber coated with a "solid phase" material is inserted into the headspace of a sample product in a sealed vessel, typically a glass vial (Figure 2). Volatile compounds in the headspace are adsorbed to the solid phase coating of the fiber. During the extraction, typically less than 60 minutes, the volatile compounds are concentrated on the solid phase. The compounds that are collected can then be desorbed into the injection port of a gas chromatograph for analysis. Collection of volatiles by headspace SPME is dependent on the equilibrium between the concentration of the volatile compound in the solid phase, the sample headspace, and the sample. Since the equilibrium of different

compounds in these three phases can be affected by several factors, SPME may not be an ideal method for all types of products. Careful selection of solid phase material, sample preparation, extraction conditions and analytical conditions can improve results obtained from SPME-GC-MS analysis of volatile compounds. Supelco provides an assortment of fibers and accessories for SPME. Because most of the published research on the use of SPME in volatile analysis of foods has been within the past five year, information regarding the use of SPME for the collection of volatile compounds in foods is still rapidly developing. SPME has been used for a wide variety of products including citrus fruits, tomatoes and edible oils.

Compound Classifiacation	Threshold (ppm)		
Hydrocarbons	90	to	2150
Substituted Furans	2	to	27
Vinyl Alcohols	0.5	to	3
1-Alkenes	0.02	to	9
2-Alkenals	0.04	to	2.5
Alkanals	0.04	to	1.0
trans,trans -2,4-Alkadienals	0.04	to	0.3
Isolated Alkadienals	0.002	to	0.3
Isolated *cis* -Alkenals	0.0003	to	0.1
trans,cis -Alkadienals	0.002	to	0.006
Vinyl Ketones	0.00002	to	0.007

Frankel 1985

Table 1.1. Sensory threshold for various classes of volatile compounds.

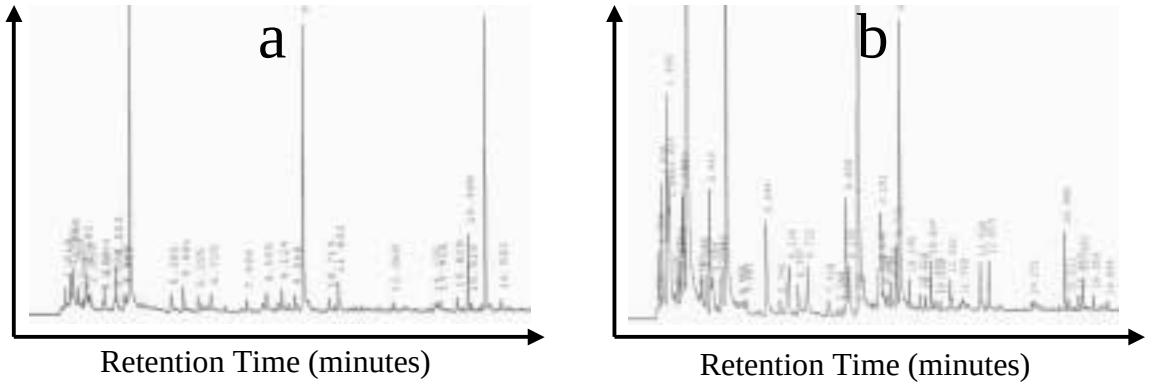


Figure 1.1. Gas chromatograph from (a) new soybean oil and (b) soybean oil that was exposed to light for 10 days at room temperature

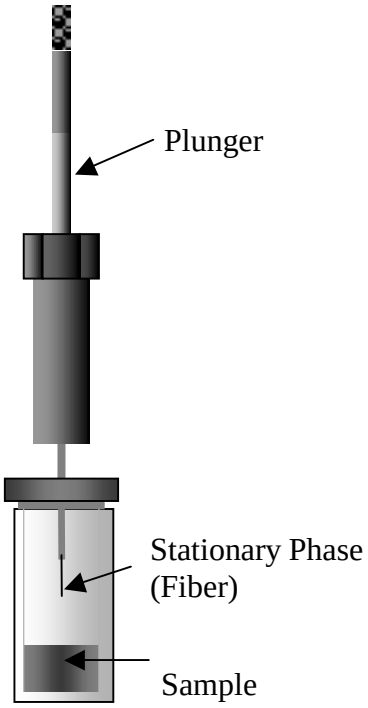


Figure 1.2. Headspace SPME

CHAPTER 2

CHEMISTRY AND REACTION OF SINGLET OXYGEN IN FOODS

Singlet oxygen is a highly reactive, electrophilic and nonradical molecule. It is different from diradical triplet oxygen in its electron arrangement. Photosensitizers can form singlet oxygen from triplet oxygen in the presence of light. The reaction rate of singlet oxygen with foods is much greater than that of triplet oxygen due to the low activation energy. Singlet oxygen oxidation produces undesirable compounds in foods during processing and storage. However, carotenoids and tocopherols in foods can minimize singlet oxygen oxidation. The in-depth scientific knowledge on the formation, reactions, quenching mechanisms and kinetics of singlet oxygen can greatly improve the quality of foods by minimizing the oxidation during processing and storage. The single oxygen oxidation of foods has contributed to the explanation of several important chemical reactions in the reversion flavor in soybean oil, sunlight flavor in milk products, the rapid losses of vitamin D, riboflavin and ascorbic acid in milk under light storage.

Introduction

The oxidation of foods causes nutritional losses, produces undesirable flavor, toxic, and color compounds, which makes foods less acceptable or unacceptable to consumers. Oxidation reactions can be formed by either diradical triplet oxygen or

nonradical singlet oxygen. The singlet oxygen can be formed in foods from triplet oxygen by photosensitized reactions. The triplet oxygen oxidation has been extensively studied during the last 70 years to improve the oxidative stability of foods and well understood through the extensive and concerted efforts of university, industry and government scientists during the last 70 years (Labuza 1971), but non-radical singlet oxygen oxidation of foods has been only studied during the last 30 years. The reasons for the lack of scientific information on the nonradical singlet oxygen oxidation in foods are (1) the study of non-radical initiated oxidation in food science has been done relatively recently, (2) the detection of short life singlet oxygen in micro seconds is difficult in complex food system, and (3) the significance and specific contributions of singlet oxygen to the oxidation of foods have not been well understood.

Most of the research on the singlet oxygen oxidation of foods has been conducted in two areas. The first is the singlet oxygen formation in foods and its functions in the production of specific compounds. The second is the study of quenching mechanisms and kinetics of quenchers to reduce singlet oxygen oxidation of foods.

The singlet oxygen oxidation of foods is very significant because of the rate of singlet oxygen oxidation is much greater than that of atmospheric triplet oxygen oxidation. Singlet oxygen rapidly increases the oxidation rate of foods even at very low temperatures (Rawls and VanSanten 1970). Singlet oxygen oxidation can produce new compounds, which are not found in ordinary triplet oxygen oxidation in foods (Frankel and others 1981; Bradley and Min 1992).

This review will discuss (1) the chemistry of singlet oxygen and its formation and reaction in foods, (2) the singlet oxygen oxidation detection in foods, (3) the chemical

reaction between singlet oxygen and lipids, proteins and vitamins, (4) the effects of singlet oxygen on the development of reversion flavor in soybean oil and sunlight flavor in milk, and (5) the rapid losses of vitamin D, riboflavin, and vitamin C in milk due to singlet oxygen, and (6) quenching mechanisms of carotenoids and tocopherols in singlet oxygen oxidation of foods.

Discoveries of oxygen and its properties

Joseph Priestley discovered oxygen in 1775 (Priestley 1775). Avogadro reported that oxygen is a diatomic molecule in 1811 (Parkes 1967). In 1848 Faraday reported that oxygen was paramagnetic and was different from other permanent gases such as nitrogen or helium gases (Parkes 1967). Mulliken (1928) reported that the paramagnetic property of oxygen is due to the parallel spins of two outer electrons of oxygen molecule. This paramagnetic uncoupled electron pair oxygen is triplet oxygen. Herzberg (1934) discovered a higher energy state of oxygen by spectroscopy and reported the oxygen as singlet oxygen. Singlet oxygen has out shell electrons paired in antiparallel spins. The importance of singlet oxygen in chemical reaction has not been recognized from 1934 to 1963. The rediscovery of singlet oxygen was made by photooxidation experiments by Foote and Wexler (1964) and Corey and Taylor (1964). The scientific progresses of singlet oxygen chemistry and their reaction with organic compounds have been made rapidly during the last 25 years. The importance of singlet oxygen reaction has been recognized in medicine, biochemistry, organic chemistry, food chemistry, environmental chemistry and organic chemistry.

Triplet Oxygen and Singlet Oxygen Chemistry

The most abundant and stable form of oxygen is triplet oxygen. Differences in the chemical properties of triplet and singlet oxygen are best illustrated by their molecular orbitals (Figure 2.1 and 2.2).

The spin multiplicity used to define spin states of molecules is defined as 2S + 1, where S is the total spin quantum number. The total spin quantum number (S) of triplet oxygen is 1. Triplet state oxygen has a spin multiplicity of 3, and is paramagnetic with diradical properties (Figure 2.1). Triplet oxygen reacts readily with other radical compounds in foods. However, most food compounds are non-radical and are in the singlet state. The triplet oxygen oxidation is initiated by the formation of radical food compound. The radical compounds react with the diradical triplet state oxygen. The molecular orbital of singlet oxygen differs from that of triplet oxygen whose electrons in the π antibonding orbital are paired, as shown in Figure 2.2. The molecule is singlet if the total spin quantum number (S) is 0 and the multiplicity of the state is 1.

Singlet oxygen is in violation of Hund's rule and a highly energetic molecule. The resulting electronic repulsion can produce five excited state conformations. The $^1\Delta$ state of singlet oxygen is responsible for most singlet oxygen oxidation in foods and is generally referred to as singlet oxygen. The most energetic π antibonding electrons of the activated $^1\Delta$ state have opposite spins and lie in one single orbital, as shown in Figure 2.2. The energy of singlet oxygen is 22.5 kcal above the ground state of triplet oxygen and exists long enough to react with other singlet state molecules (Korycka-Dahl and Richardson 1978; Girotti 1998). Singlet oxygen, which is not a radical compound, reacts with non-radical, singlet-state and electron-rich compounds containing double bonds.

The lifetime of singlet oxygen is from 50 to 700 microseconds, depending upon the solvent system of foods. The reaction temperature has little effect on the oxidation rate of singlet oxygen with foods due to the low activation of 0 to 6 kcal/mole (Yang and Min 1994). A summary of chemical properties of singlet and triplet oxygen is shown in Table 2.1.

Singlet oxygen can directly react with electron rich double bonds without the formation of free radical intermediates. Singlet oxygen oxidation reaction is very rapid in foods due to the low activation energy required for the chemical reaction. The reaction rates of singlet oxygen and triplet oxygen with linoleic acid are 1.3×10^5 $M^{-1}Sec^{-1}$ and 8.9×10^1 $M^{-1}Sec^{-1}$, respectively (Rawls and VanSanten 1970; Doleiden and others 1974). The importance of singlet oxygen to food quality has been recognized during the past 30 years, especially concerning lipids and vitamins, which are typically the most sensitive to oxidative damage.

Singlet Oxygen Formation

Singlet oxygen can be formed chemically, enzymatically and photochemically as shown in Figure 2.3 (Krinsky 1977). Some of the mechanisms for singlet oxygen formation in Figure 2.3 have not been unequivocally proven scientifically and have been questioned.

Detailed studies of the many different mechanisms for the formation of singlet oxygen in foods should be studied further. Photosensitizers such as chlorophyll, pheophytins, porphyrins, riboflavin, myoglobin and synthetic colorants in foods can absorb energy from light and transfer it to triplet oxygen to form singlet oxygen (Foote

and Denny 1968; Afonso and others 1999; Lledias and Hansberg 2000). The chemical mechanism for the formation of singlet oxygen in the presence of sensitizer, light and triplet oxygen (Figure 2.4) is mainly responsible for singlet oxygen formation in foods. The photosensitizer absorbs the ultraviolet or visible radiation energy rapidly and becomes an unstable, excited, singlet-state molecule ($^1Sen^*$). The excited singlet photosensitizer loses its energy by internal conversion, emission of light, or intersystem crossing as shown in Figure 2.4.

Internal conversion involves the transformation from high energy to less energy state by releasing energy as heat. Emission of fluorescence converts the excited singlet sensitizer to ground state singlet sensitizer. The excited sensitizer may also undergo an intersystem crossing from the excited singlet-state molecule to an excited triplet-state molecule ($^3Sen^*$). The emission of phosphorescence converts the excited triplet sensitizer to ground state singlet sensitizer. The lifetime of the $^3Sen^*$ is greater than $^1Sen^*$. The $^3Sen^*$ reacts with $^3O_2$ to form $^1O_2$ and ^1Sen by triplet-triplet annihilation mechanism. The sensitizer returns to ground state (^1Sen) and may begin the cycle again to generate singlet oxygen. Sensitizers may generate 10^3 to 10^5 molecules of singlet oxygen before becoming inactive (Kochevar and Redmond 2000).

Type I and Type II Pathways

The $^3Sen^*$ may react directly with compound (RH) such as linoleic acid or phenol compounds by donating and accepting hydrogen or electron and produces free radicals or free radical ions as shown in Figure 2.5. This mechanism is known as Type I pathway (Gollnick 1968; Foote 1976; Sharman and others 2000). The $^3Sen^*$ acts as a

photochemically activated free radical initiator for R· formation. The R· can abstract hydrogen from other compounds to initiate the free radical chain reaction. The radical compound (R·) reacts with radical triplet oxygen to form peroxy radical. The $^3Sen^*$ in Type I pathway can also react with $^3O_2$ to form superoxide anion by electron transferring to triplet oxygen. Less than 1% of the reaction of triplet sensitizer and triplet oxygen produces superoxide anion (Kepka and Grossweiner 1972). The rate of the Type I pathway is mostly dependant on the type and concentration of sensitizers and substrate compound. Readily oxidizable compounds such as phenols and reducible compounds such as quinines favor Type I pathway (Korycka-Dahl and Richardson 1978).

The excited triplet sensitizer ($^3Sen^*$) may react with $^3O_2$ to form $^1O_2$ and singlet sensitizer by triplet sensitizer-triplet oxygen annihilation in Type II pathway (Figure 5). Energy is transferred from the high energy excited triplet sensitizer to low energy triplet oxygen to form high energy singlet oxygen and low energy ground state singlet sensitizer (Sharman and others 2000). More than 99 % of the reaction between triplet sensitizer and triplet oxygen produce singlet oxygen (Kepka and Grossweiner 1972). The rate of Type II pathway is mostly dependant on the solubility and concentration of oxygen in the food system. As the oxygen in a system becomes depleted, the shift from Type II to Type I mechanism is favored (He and others 1998; Song and others 1999). Oxygen is more soluble in nonpolar lipids than it is in water (Ke and Ackman 1973). The chlorophyll induced singlet oxygen formation in soybean oil favors the Type II pathway. In contrast, water-based food systems such as milk favor Type I pathway due to the reduced availability of oxygen. The shift from Type I to Type II or vice versa is dependent on the concentration of oxygen and the types and concentration of compound.

Types I and II reactions will enhance oxidation by either the formation of reactive radical compound species or the production of singlet oxygen. The competition between compound and triplet oxygen for the excited triplet sensitizer largely determines whether the reaction pathway is Type I or Type II. Photosensitized oxidation may change the types of pathway during the course reaction as the concentration of compound and oxygen changes. In aqueous-lipid biphasic systems, the longer half-life of singlet oxygen in the lipid phase favors Type II pathway.

Detection and Evaluation of Singlet Oxygen in Foods

The singlet oxygen detection during photosensitized oxidation of foods is difficult due to the short lifetime. However, analytical techniques have been developed for the detection of singlet oxygen and the measurement of activity. One of the most common detection methods is electron spin resonance spectroscopy (ESR), which is highly sensitive for the detection of free radicals. A spin trapping agent such as 2,2,6,6-tetramethly-4-piperidone (TMPD) can react with singlet oxygen to form a stable nitroxide radical adduct, 2,2,6,6-tetramethyl-4-piperidone-*N*-oxyl (TAN) which is measured by ESR (Sharman and others 2000). The reaction of 2,2,6,6-tetramethyl-4-piperidone with singlet oxygen for 2,2,6,6-4-piperidone-N-oxyl formation is shown in Figure 2.6.

Although other reactive oxygen species such as superoxide and hydroxyl radicals can react with TMPD, they do not convert TMPD to TAN. This method is highly specific to singlet oxygen (Ando and others 1997). Ando and others (1997) further confirmed this specificity by observing an inhibition of TAN formation upon the addition

of two known singlet oxygen scavengers such as sodium azide and histidine and not upon the addition of a hydroxyl radical scavenger such as dimethyl sulfoxide or a superoxide scavanger such as superoxide dismutase. ESR detected the formation of singlet oxygen in meat (Whang and Peng 1988) and milk (Bradley 1991) using a spin trapping technique. Effects of 0, 5 and 15 minutes illumination on electron spin resonance spectrum of 2,2,6,6-4-piperidone-N-oxyl radical in water solution of riboflavin and 2,2,6,6-tetramethyl-4- piperidone is shown in Figure 2.7 (Min and Lee 1996). The use of different spin trapping compounds may be utilized to differentiate reactive oxygen species by ESR. He and others (1997) used ESR to detect superoxide and hydroxyl radicals using 5,5-dimethyl-1-pyrroline-*N*-oxide as a spin trapping agent.

Singlet oxygen is detected by its chemiluminescence at 1270 nm (Kanofsky and Axelrod 1986; Macpherson and others 1993; Darmanyan and Jenks 1998). The energy differential between singlet oxygen and ground state oxygen can be released as a 1270 nm photon, which is very specific to singlet oxygen. The spin change transition, which is unfavorable, produces the desired light emission. Singlet oxygen is detected by measuring the emission at 1270 nm in biological systems using a solid-state near-infrared detector. Kanofsky (2000) described this singlet oxygen detection analytical method in detail.

Two relatively simple spectrophotometric methods can be used to measure singlet oxygen production from a photosensitizer. 1,3-Diphenylisobenzofuran is soluble in organic solvents and can be measured at 410 nm absorbance decrease as the molecule reacts directly with singlet oxygen (Kochevar and Redmond 2000). *p*-Nitrosodimethylalanine is soluble in aqueous systems and can be monitored at 440 nm

absorbance decrease as the molecule reacts with an imidazole intermediate (Kochevar and Redmond 2000). The rate of singlet oxygen formation is proportional to the slope of the loss of absorption versus light irradiation time.

Cholesterol reacts with singlet oxygen to form specific oxidative products via the "ene" reaction. This specificity makes the use of cholesterol as an effective indicator of singlet oxygen oxidation *in situ*, where the use of other detection techniques is difficult. The reaction of cholesterol with singlet oxygen produces 3β-hydroxy-5α-cholest-6-ene-5-hydroperoxide (5α-OOH), 3β-hydroxycholest-4-ene-6α-hydroperoxide (6α-OOH), and 3β-hydroxycholest-4-ene-6β -hydroperoxide (6β -OOH) as shown in Figure 2.8. The reaction of cholesterol with triplet oxygen produces 3β-hydroxycholest-5-ene-7α -hydroperoxide (7α -OOH) and 3β-hydroxycholest-5-ene-7β -hydroperoxide (7β -OOH) (Girotti 1998; Girotti and Korytowski 2000). The specificity of cholesterol with triplet oxygen and singlet oxygen can differentiate between singlet oxygen initiated reaction and triplet oxygen initiated reaction. Girotti and Korytowski (2000) described in detail on the use of cholesterol as an indicator of singlet oxygen.

Azide and histidine are commonly used to determine the singlet oxygen oxidation of compounds as these agents act as quenchers of singlet oxygen and greatly suppress the activity of singlet oxygen and the consumption of singlet oxygen (Song and others 1999). Singlet oxygen quenching by azide is thought to be a charge transfer process in which molecular triplet oxygen is released after reaction; therefore no oxygen is consumed. Oxygen consumption can be easily assessed (Telfer and others 1994). Enhancement of oxygen consumption in deuterium oxide, relative to water, is often used as an indicator of the involvement of singlet oxygen in oxidation due to the increased half-life of singlet

oxygen in deuterium oxide (Athar and others 1988; Pecci and others 2000). Further differentiation may be accomplished by using specific quenchers. Several quenching agents have specificity towards reactive oxygen species. Histidine, β-carotene, ascorbic acid, sodium azide and 2,5-dimethyl furan are quenchers for singlet oxygen, superoxide dismutase is a superoxide anion quencher.

Current singlet oxygen detection methods under investigation are quantitative determination using laser deflection calorimetry (Schneider and others 2000) and time-resolved singlet oxygen detection (Nonell and Braslavsky 2000). Laser deflection calorimetry is very selective in the determination of singlet oxygen in heterogeneous systems (Schneider and others 2000). Future advances in time-resolved singlet oxygen detection should include the detection of photosensitized and non-photosensitization production of singlet oxygen in heterogeneous system such as food (Nonell and Braslavsky 2000). Recently, Andersen and Ogilby (2001) recorded the time-resolved absorption spectrum of singlet oxygen using transmission microscopy.

Triplet Oxygen and Singlet Oxygen Oxidation with Fatty Acids

The oxidation rate of food is dependant on several factors including temperature, the presence of inhibitors or catalysts, nature of the reaction environment and the nature of the compounds (Frankel 1985). These factors are important in varying degrees to both singlet and triplet oxygen oxidations in foods. Temperature has little effect on singlet oxygen oxidation, but has a significant effect on triplet oxygen oxidation, which requires high activation energy. Polyunsaturated fatty acids are more susceptible to radical initiated triplet oxygen oxidation than monounsaturated fatty acids, a property that is

primarily due to the lowered activation energy in the initiation of free radical formation in polyunsaturated fatty acids compared to monounsaturated fatty acids (Lea 1952). The type of polyunsaturated fatty acids is not important in singlet oxygen oxidation. The total numbers of double bonds is more important in singlet oxygen oxidation than the types of double bonds such as non-conjugated or conjugated dienes or trienes, which are important in free radical triplet oxygen oxidation.

Electrophilic singlet oxygen is seeking electrons to fill its highest degenerate vacant molecular orbital. One of the most important reaction characteristics of singlet oxygen is that it can directly react with the electron-rich double bonds of unsaturated molecules (Adam 1975; Beutner and others 2000).

Singlet oxygen participates in reactions such as 1,4-cyclo addition to diene and heterocyclic compounds, the "ene" reaction and 1,2-cycloaddtion to olefins, all of which involve direct reaction with double bonds as shown in Figure 2.9 and 2.10. Singlet oxygen reaction with linoleic or linolenic acid forms both conjugated and nonconjugated diene hydroperoxides (Figure 2.10). The linoleic and linolenic acid reactions with triplet oxygen produces only conjugated diene hydroperoxides. Direct reaction of singlet oxygen with double bonds permits the formation of hydroperoxides at positions 10 and 12 in linoleic acid and 10 and 15 in linolenic acid, which do not form in triplet oxygen oxidation (Frankel and others 1979). These properties may be used to determine singlet oxygen activity in lipids (Stratton and Lieber 1997) and produces compounds that are absent in triplet oxygen oxidation.

The reaction rates of singlet oxygen with oleic, linoleic, linolenic, and arachidonic acids are 0.74, 1.3, 1.9, and 2.4 x 10^5 $M^{-1}Sec^{-1}$, respectively, which is relatively

proportional to the number of double bonds in the molecules instead of types of double bonds, such as conjugated or nonconjugated double bonds (Doleiden and others 1974). Reactivity increases as the ionization energy decreases due to the presence of alkyl groups adjacent the C=C double bond. However, steric hindrance to the double bond will lower the reactivity (Beutner and others 2000).

Diradical triplet oxygen reacts with radical food compounds. However, food compounds are not radical compounds. The initiation of radical formation in food is at the carbon which requires least energy for a hydrogen atom removal (Frankel 1985). The removal of hydrogen from a saturated fatty acid requires approximately 100 kcal/mol of energy. The energy required for the removal of hydrogen at different carbons of linoleic acid is quite different as is shown in Figure 2.11. Hydrogen at position 2.11 of linoleic acid is most easily removed due to the presence of a double bond on both sides, requiring only about 50 kcal/mol.

Once the hydrogen is removed, a pentadienyl radical intermediate between carbon 9 and carbon 12 of linoleic acid is formed (Figure 2.12). The pentadienyl radical provides an equal mixture of conjugated 9- and 13- diene radical and produces 9- and 13-conjugated diene hyroperoxides upon reaction with triplet oxygen, as shown in Figure 2.12.

Triplet oxygen autoxidation produces only the conjugated diene hydroperoxides in linoleic and linolenic acids. The relative reaction ratios of triplet oxygen with oleic, linoleic and linolenic acid for hydroperoxide formation are 1**:**12**:**25, which is dependent upon the relative difficulty for the radical formation in the molecule (Min and others 1989). The reaction rate of triplet oxygen with linolenic acid is about twice as fast as that

of linoleic acid because linolenic acid has 2 pentadienyl groups in the molecule, compared to the linoleic acid with one pentadienyl group. Neff and Frankel (1980) reported the hydroperoxides formed by triplet oxygen and singlet oxygen with oleic, linoleic and linolenic acids as shown in Table 2.2.

Singlet oxygen oxidation produces hydroperoxides at the positions of double bonds without migration. The relative reaction rates of triplet oxygen and singlet oxygen with oleic, linolec and linoleic acids are shown in Table 2.3. The reaction rates of triplet oxygen and singlet oxygen with linoleic acid are 8.9×10^{1} $M^{-1}Sec^{-1}$ and 1.3×10^{5} $M^{-1}Sec^{-1}$, respectively (Rawls and VanSanten 1970). That is, the reaction rates of singlet oxygen reacts with linoleic acid about 1450 times faster than that of the triplet oxygen with linoleic acid.

Soybean oil contains 1.0 to 1.5 ppm of chlorophyll (Brekke 1980), which is an excellent singlet oxygen sensitizer. Min and Lee (1988) reported that headspace volatile compounds of soybean oil increased as added chlorophyll increased from 0, 2, 4 and 6 ppm to 8 ppm. Soybean oil containing no chlorophyll, which was removed by silicic acid liquid column chromatography, did not produce headspace volatile compounds under light under the identical experiment condition at 10 ^{o}C. However, the effects of 0, 2, 4, 6 and 8 ppm added chlorophyll did not have any effect on the formation of volatile compounds of soybean oil under dark storage. The formation of headspace volatile compounds in the soybean oil decreased inversely with the amount of added β-carotene, which quenches singlet oxygen (Lee and Min 1990). The very rapid formation of volatile compounds in the soybean oil in the presence of chlorophyll, light and oxygen was due to the singlet oxygen oxidation.

Reversion Flavor in Soybean Oil

Soybean oil represents about 70% of all edible fats and oils consumed in the United States (Golbitz 2000). Soybean oil is inexpensive and widely available compared to other edible oils. The development of reversion flavor, described as beany or grassy, is a unique defect to soybean oil and can be formed in soybean oils, which have low peroxide values (Ho and others 1978). To improve the flavor stability and quality of soybean oil, reversion flavor has been extensively studied in soybean oil since 1936 (Ho and others 1978).

Smouse and Chang (1967) identified 2-pentyl furan in reverted soybean oil and reported that it significantly contributed to the reversion flavor of soybean oil. Chang and others (1983) isolated and identified all four 2-pentenyl-furan isomers in reverted soybean oil.

Sensory evaluation showed that the addition of 2-ppm 2-pentyl furan to freshly deodorized and bland soybean oil produced the "reverted" soybean oil flavor. The addition of 2-ppm 2-pentyl furan to deodorized cottonseed oil and corn oil also produced reversion flavor found in reverted soybean oil (Chang and others 1966). Ho and others (1978) reported that 2-(1-pentenyl) furan contributed to reversion flavor. Smagula and others (1978) reported that 2-(2-pentenyl) furan is also a contributor according to sensory evaluation. Flavor thresholds of the 2-pentenyl furan isomers were between 0.25 and 6 ppm.

Smouse and Chang (1967) and Ho and others (1978) proposed the mechanisms for the formation of 2-pentyl furan from linoleic acid and 2-pentenyl furan isomers from

linolenic acid using triplet oxygen, respectively. The proposed mechanisms for the formation of 2-pentyl furan from linoleic acid and isomers of 2-pentenyl furan from linolenic acid by triplet oxygen have been questioned. The formations of both the 2-pently furan and 2-pentenyl furan require a hydroperoxide at carbon 10 of linoleic acid. The formation of 10-hydroperoxide in linoleic or linolenic acids by free radical triplet oxygen oxidation is highly improbable, but is very common in the singlet oxygen oxidation of linoleic or linolenic acids as shown in Table 2.2. Min (2000) reported that the chemical mechanisms for the formations of 2-(2-pentenyl) furan from linoleic acid and 2-pentyl furan formation from linolenic acid using singlet oxygen as shown in Figures 2.13 and 2.14 respectively.

Callison (2001) identified 2-pentylfuran and 2-pentenyl furan in soybean oil containing 5-ppm chlorophyll b during storage under light for 96 hours. The formation of 2-pentyl and 2-pentenyl furan increased with increasing storage time and concentration of chlorophyll as shown in Table 2.4. 2-Pentyl furan and 2-pentenyl furan were formed only in the presence of light and chlorophyll in soybean oil. Soybean oil containing 5-ppm chlorophyll did not produce 2-pentyl furan and 2-pentenyl furan during dark storage. The chlorophyll free soybean oil obtained by silicic acid chromatography did not produce either 2-pentyl furan or 2-pentenyl furan during light storage. The singlet oxygen oxidation reaction is involved for the formation of 2-pentyl furan and 2-pentenyl furan, which have been reported to be mainly responsible for reversion flavor in soybean oil. The chlorophyll, which is an excellent photosensitizer, in soybean oil should be carefully removed during the oil processing to minimize the formation of reversion during storage.

Singlet Oxygen Oxidation with Proteins

The major research in singlet oxygen oxidation in foods has been involved with lipids. Most of the research in singlet oxygen oxidation of proteins has been done in the medical and health fields. Singlet oxygen reacts primarily with five amino acids, which are tryptophan, histidine, tyrosine, methionine and cystein to form peroxides (Michaeli and Feitelson 1995; 1997). Reactions with methionine, histadine and tyrosine are shown in Figure 2.15, 2.16 and 2.17. The reaction rate of proteins and singlet oxygen is mostly dependant on the number and types of amino acids, which have double bonds or electron rich sulfur atom (Michaeli and Feitelson 1994).

Other amino acids react with singlet oxygen at two to three orders of magnitude lower than tryptophan, histidine, tyrosine, methionine or cystein (Michaeli and Feitelson 1995). Tryptophan, histidine and tyrosine contain double bonds, can readily react with eletrophilic singlet oxygen. Methionine and cystein contain a sulfur atom with 4 nonbonding electrons and reacts rapidly with the electrophilic singlet oxygen. As amino acids become altered by singlet oxygen, the protein or enzyme are denatured, looses functionality and can aggregate. The information on the protein singlet oxygen oxidation has not been easily available. The quenching effect of various amino acids and the use of histidine in several analytical techniques involving singlet oxygen have been reported (Song and others 1999). The role of methionine in the sunlight flavor of milk will be discussed later.

The reaction rate of singlet oxygen oxidation of protein is dependant on pH, temperature, and dielectric constant of the medium and presence of the five singlet

oxygen reactive amino acids, which are tryptophan, histidine, tyrosine, methionine and cystein.

Bisby and others (1999) reported that pH influences the singlet oxygen oxidation of N-acetyl tyrosine ethyl ester and amino acids. The reaction rates of these compounds may be deprotonation states of these compounds. Bisby and others (1999) reported that singlet oxygen reaction rate constant (k_t) by histidine is greatest when the pH >> pKa (5×10^7), compared to 10^6 when pH << pKa. Fully protonated histidine had a k_t of $\leq 10^4$. Tryptophan, having no ionizable protons in the ionizable groups in the tested pH range, showed no pH effect. Their research also showed that deprotonation of phenols in tyrosine results in an increase in k_t.

Michaeli and Feitelson (1995) compared the singlet oxygen reaction rate of large peptides to a solution of a comparative concentration of amino acids to each respective peptide. The free amino acids had a higher oxidation rate than did the native peptide. The fully denatured peptide had a quenching rate equivalent to the comparative solution of free amino acids, which indicates that the availability of amino acids and not the peptide bond is an important factor to the reactivity of a protein. The tertiary structure of the proteins acts to inhibit the reaction of singlet oxygen with singlet oxygen reactive amino acids, thereby imparting some degree of protection.

The reaction between amino acids and singlet oxygen is solvent dependant (Miskoski and Garcia 1993). Some amino acids can act as both physical and chemical quenchers. The singlet oxygen reaction rate constant of proteins is dependant on the types of amino acids, their accessibility to singlet oxygen and the dielectric constant of the medium (Michaeli and Feitelson 1994). Jung and others (1995) reported that

histidine and tyrosine accelerated the riboflavin-sensitized destruction of ascorbic acid and suggested that intermediate products of amino acid and singlet oxygen were accelerating the oxidation of ascorbic acid.

Singlet Oxygen Oxidation of Amino Acid for Sunlight Flavor in Milk

Light-induced off-flavor in milk, which is referred to as sunlight flavor, has long been a recognized problem in the milk industry. White and Bulthaus (1982) reported that 53 of 90 grocery store milk samples in plastic jugs under light have characteristic light induced off flavor. The mechanisms for the formation of sunlight flavor in milk have been studied for more than 50 years.

Many studies have identified several sulfur compounds in light-exposed milk. Samuelson (1962) used sulfur-35 radioisotope-labeled milk and found that mercaptan, sulfides and dimethyl sulfides increased in milk during light irradiation. Harper and Brown (1964) found that photooxidation of cysteine produced mercaptan, sulfide and disulfide. However, Patton and Josephson (1953) reported that cystine and cysteine were not necessary for the development of sunlight flavor in milk. Patton (1954) determined that both riboflavin and methionine were necessary for the formation of sunlight flavor. Allen and Parks (1975) reported that sunlight flavor development originated from a non-protein source, likely free amino acids. Harper and Brown (1964) and Allen and Parks (1975) agreed that methional seems to be an important compound in the development of sunlight flavor, which was postulated to develop from methionine, although several researchers had difficulties in isolating and identifying methional. Balance (1961) reported that methional decomposed to form methyl mercaptan and dimethyl disulfide,

perhaps lending to the difficulty of isolating methional. Allen and Parks (1975) were able to identify methional in skim milk exposed to light. They found that the sunlight flavor would develop in a serum obtained by negative pressure dialysis, indicating a nonprotein source. Hoskin (1979) postulated the formation of methional from methionine by a Strecker degradation-like reaction in the presence of riboflavin (Figure 2.18). However, the sunlight flavor is not produced under dark where Strecker degradation-like reaction can be occurred under dark. If methional is formed from methionine by Strecker degradation-like reaction under dark as postulated in Figure 2.18, the milk should produce sunlight flavor. The postulation of methional formation from methionine by a Strecker degradation-like reaction may be questionable.

Forss (1979) postulated that methanethiol, dimethyl sulfide and dimethyl disulfide formed in light exposed milk and was responsible for sunlight flavor. Dimick (1982) reported that prolonged light-exposure altered the methional flavor to a methyl mercaptan-like flavor, and supported Balance's findings that methional decomposed to methyl mercaptan and dimethyl disulfide. Dimick and Kilara (1983) identified methionine sulfoxide, which was derived from methionine in the presence of light and concluded that riboflavin, protein and oxygen were required for the development of light-induced sunlight flavor in milk.

Foote (1976) was the first to suggest the role of singlet oxygen in light-induced off-flavor and reported that methionine sulfoxide is a product of singlet oxygen oxidation of methionine. Singlet oxygen formation in milk exposed to sunlight was detected using electron spin resonance spectroscopy (Bradley 1991). Jung and others (1998) reported that a trained sensory panel could identify sulfurous off-flavors in milk that was exposed

to sunlight for 15 minutes. No sunlight flavor was detected in milk after a period of 8 hours when the samples were stored in the dark. Sensory panel evaluations concluded that dimethyl disulfide was a key compound for the light-induced sunlight flavor in milk. Jung and others (1991) reported the mechanism for the formation of dimethyl disulfide by reaction between singlet oxygen and methionine as shown in Figure 2.19.

Jung and others (1998) evaluated light-induced off-flavor in solutions of cysteine, methionine or valine exposed to sunlight. A hydrogen sulfide odor was found in the light-exposed cystein sample and dimethyl disulfide was identified in the methionine sample that was exposed to light. Similar treatment of valine produced no such odors. No such odors were found in the absence of either light or riboflavin, thereby supporting the theory that light and riboflavin are required for the development of light-induced off-flavor. The riboflavin in milk was removed by liquid column chromatography using Fluorosil as a stationary phase. The riboflavin-free milk did not produce dimethyl disulfide or sunlight flavor under light storage. The sunlight flavor in milk was mainly due to the singlet oxygen oxidation of methionine in milk. The formation of dimethyl disulfide in milk decreased as the amount of added ascorbic acid increased from 0, 200, 500 ppm to 1000 ppm. Ascorbic acid which is a singlet oxygen quencher minimized the formation of dimethyl disulfide in milk as was expected (Jung and others 1998).

Singlet Oxygen Oxidation of Meats

Myoglobin and other hemoproteins have been identified as the primary photosensitizers of lipid oxidation in meats (Whang and Peng 1988). Both myoglobin and oxymyoglobin have the ability to absorb light energy, resulting in the formation of

metmyoglobin through a photooxidative reaction. Proteins are susceptible to oxidation, producing a variety of flavor precursors (King 1996). Whang and Peng (1988) reported that peroxide values for pork and turkey meat exposed to light were significantly higher than those stored in the dark. Furthermore, they found that myoglobin and its derivatives functioned as photosensitizers in model systems for the formation of singlet oxygen. Red No. 3 in processed meats was also found to be a singlet oxygen sensitizer (Usuki and others 1984). The presence of erythrosine, which is a photosensitizer for singlet oxygen formation, accelerated the deterioration of flavor in luncheon meat during light exposure (Chan 1975). The in-depth and detailed effects of singlet oxygen on the flavor stability and quality of meat products should be studied.

Singlet Oxygen Reaction with Carbohydrates

Carbohydrates are relatively insensitive to singlet oxygen oxidation, particularly in the presence of more reactive materials. However, secondary products and radical species generated from singlet oxygen activity could be problematic in further reacting with carbohydrates. Due to the low reactivity of carbohydrates with singlet oxygen, little has been published on this subject.

Singlet Oxygen Oxidation of Vitamin D

Vitamin D is important for normal mineralization and growth of bones. It acts as a pheromone which, when hydrolyzed into 1, 25-dihydroxy vitamin D, stimulates absorption of calcium in the intestine. Milk is an important source of calcium for growing children and is typically fortified with vitamin D at a level of 400 IU per quart;

however, it can be rapidly destroyed under light storage in display cases (United States Public Health Services/Food and Drug Administration 1993). Rinken and Watherson (1993) reported that vitamin D in an acetonitrile system, which does not contain riboflavin, was not destroyed under light, but vitamin D in skim milk which contains riboflavin was destroyed during light storage. King and Min (1998) reported that the oxidation of vitamin D in a model system did not occur in the absence of either riboflavin or light. They systematically studied the effects of light, riboflavin, and oxygen on photo-activated singlet oxygen oxidation of vitamin D. The oxidation of vitamin D in a model system of 12% water, 88% acetone and 15 ppm riboflavin under light or dark was studied by measuring the headspace oxygen remaining in a gas-tight sample bottle. Riboflavin accelerates the oxidation of vitamin D by singlet oxygen under light, but did not affect vitamin D oxidation in darkness. King (1996) separated an oxidized compound of vitamin D by high pressure liquid column chromatography and identified it as vitamin D-5,6 epoxide by a combination of mass, infrared and ultraviolet spectrometry. King Singlet oxygen can react with the conjugate triene structure of vitamin D, forming vitamin D-5,6 epoxide as shown in Figure 2.20. Light activates riboflavin, which subsequently results in the formation of singlet oxygen from triplet oxygen. The rate of singlet oxygen formation by 15-ppm riboflavin in a model system was 7.14 micromoles/mL headspace-hour (Li and others 2000). The reaction rate of vitamin D with singlet oxygen was 2.2×10^7 $M^{-1}Sec^{-1}$. The rate of headspace oxygen depletion is dependant on the amount of riboflavin or vitamin D (Li and Min 1998). The addition of 15-ppm riboflavin has a significant effect on the loss of vitamin D under light. The absence of either riboflavin or light resulted in no vitamin D loss in milk system.

Singlet Oxygen Oxidation of Riboflavin

Milk contributes 40 to 50% of total dietary riboflavin in the United States and many other western nations. It is very well known that riboflavin in milk is easily destroyed in the presence of light. Allen and Parks (1979) reported that as the sunlight flavor in milk developed, the riboflavin was destroyed. The destruction of riboflavin in skim milk and whole milk is a first order reaction in relation to the exposure time to light (Allen and Parks 1979). Riboflavin in food is relatively stable under cooking conditions and also stable in milk packaged in cartons or dark bottles to protect from light. Milk exposed to sunlight for 30 minutes lost 30% riboflavin whereas only a 12% loss occurred in milk boiled for the same time period (Wishner 1964). Herreid and Ruskin (1952) reported the loss of riboflavin as high as 80% dependant on the type of container during storage under light or cooking or both.

Riboflavin is a good singlet oxygen sensitizer for the formation of singlet oxygen under light (Bradley and Min 1992). Riboflavin has a triene structure and many double bonds that can react with singlet oxygen (Bradley and Min 1992). The riboflavin content in milk in a gas-tight bottle under light storage decreased as the headspace oxygen decreased in the bottle, which suggests that the riboflavin decrease was due to oxidation. When the milk was purged with nitrogen to remove the oxygen content in a sample bottle and air tightly sealed, the riboflavin content of the nitrogen-gas-purged milk was much higher than that of the milk without nitrogen purging for 2 days under light. However, the samples with and without nitrogen packaging stored under dark for 2 days had the same amounts of riboflavin. This result also suggests that the rapid riboflavin loss in

milk during light storage was due to singlet oxygen oxidation of riboflavin. Singlet oxygen can be formed under light, but not under dark in the presence of riboflavin. Exposure of milk to sunlight has been shown to be more detrimental to riboflavin loss than exposure to fluorescent light. Losses from exposure to light are dependant upon the light intensity, wavelength, exposure time and package. Table 2.5 lists losses of riboflavin over time during exposure to sunlight dependant on the packaging material. Dunkley and others (1962) reported riboflavin losses of 0.1-0.4 mg/L/h in milk in clear glass containers under fluorescent light. Maniere and Dimick (1976) reported that the free form of riboflavin, which constitutes 82% of the total riboflavin, is most labile to light. Light exposure to 450 nm, which corresponds to the maximum absorption of riboflavin, was most destructive to riboflavin.

Singlet Oxygen Oxidation of Ascorbic Acid

Exposure of milk to sunlight can result in 80-100% losses of ascorbic acid after only 60 minutes as shown in Table 2.5 (Satter and deMan 1975). The 80-90% of ascorbic acid may be lost to 24-hour fluorescent light exposure of 400-550 nm corresponding to maximum absorbance of riboflavin. Yang (1994) and Jung and others (1995) studied the effect of singlet oxygen on the stability of ascorbic acid in a model system. The loss of ascorbic acid increased as the added riboflavin content in milk increased under light storage. The 100% of ascorbic acid was destroyed after 12 minutes light exposure when 6-ppm riboflavin was added. Whereas only 2% was destroyed in the sample to which no riboflavin was added Jung and others (1995). The loss of ascorbic acid was significantly reduced when sodium azide, which is a singlet oxygen quencher,

was added to the sample. Jung and others (1995) reported that the reaction rate of ascorbic acid with singlet oxygen was 5.77 x 10^8 $M^{-1}Sec^{-1}$ in a potassium buffer at pH 6 and 20 ^oC. As the solution pH decreased from 7.5 to 4.5 the reaction rate of ascorbic acid with singlet oxygen decreased. These results were further supported by Bisby and others (1999) who reported that ascorbate ion was more reactive towards singlet oxygen than was its protonated form. Yang (1994) reported a reaction rate for ascorbic acid with singlet oxygen of 3.08 x 10^8 $M^{-1}Sec^{-1}$ in an aqueous solution of pH 7 and 25 ^oC. This suggests that the rapid loss of ascorbic acid in milk under light storage was due to singlet oxygen, which was produced by riboflavin under light.

Quenching Mechanisms

Singlet oxygen is highly reactive towards bioorganic molecules that have π electrons or lone pairs of low ionization energy. The quenching agent is altered into a vibrational or electronic excited state in physical quenching. Chemical quenching involves the reaction between the quenching agent and singlet oxygen. Intermediates such as exciplexes, diradicals or zwitterions are commonly involved in quenching reactions (Beutner and others 2000).

Other than exclusion of light and reduction of oxygen present, the use of quenching agents is perhaps the best way to reduce singlet oxygen oxidation. Natural food components such as tocopherols, carotenoids and ascorbic acid can act as effective singlet oxygen quenchers (Li and others 2000). The effectiveness of quenching agents has been mainly studied in either liquid products or in aqueous model solutions, very often homogeneous. The determination of the presence of singlet oxygen and its

subsequent reactions become much more complicated in heterogeneous systems and in tissues. Fukuzawa (2000) lists several factors that should be considered when evaluating quenching agents in membranes, such as the mobility of the quenching agent in membranes, partitioning and activity in polar versus non-polar regions and the solubility of singlet oxygen in the membrane.

Quenching agents may be involved with to minimize the development or activity of singlet oxygen at several stages in the oxidation of foods. Figure 2.21 shows the development of singlet oxygen and its subsequent reaction with compound (A) to from the oxidized product (AO_2). At every stage in this reaction there is at least one alternate route, which, if taken, would minimize the oxidation of the compound (A). The first step represents the return of the excited singlet sensitizer ($^1Sen^*$) to ground state (^1Sen) without intersystem crossing to form the excited triplet sensitizer ($^3Sen^*$). The second represents reaction with a quenching agent (Q) at a rate represented as k_Q, returning the excited triplet sensitizer ($^3Sen^*$) to ground state (^1Sen) prior to reaction with triplet oxygen. The excited triplet sensitizer ($^3Sen^*$) may react with triplet oxygen ($^3O_2$) to form singlet oxygen ($^1O_2$). Following its creation, there are three fates for singlet oxygen in foods. (1) It may naturally decay to the ground state at a rate represented as k_d. (2) It may react with a singlet-state compound (A) at a rate represented as k_r forming the oxidized product AO_2. (3) It may be destroyed by a quenching agent by either combining with the quencher, at a rate represented as $k_{ox\text{-}Q,}$ to form the product QO_2 or by passing its energy to the quenching agent and returning to free triplet oxygen, at a rate represented as k_q.

As represented by Figure 2.21, there are three points at which a quenching agent may act, one is quenching of the excited triplet sensitizer and the other two are quenching of singlet oxygen by either chemical or physical means. Chemical quenching involves reaction of singlet oxygen with the quenching agent to produce an oxidized product (QO_2). Physical quenching results in the return of singlet oxygen to triplet oxygen without the consumption of oxygen or product formation achieved by either energy transfer or charge transfer. Therefore, triplet oxygen quenchers must either be able to donate electrons or to accept energy 22.5 kcal above ground state. An example of the latter is β–carotene that has a low singlet energy state and can therefore accept the energy from singlet oxygen (Lee and Min 1990). Ascorbic acid is an example of a chemical that can quench the excited sensitizer. Table 6 lists quenching rates of several antioxidants.

Quenching Mechanism of Carotenoids

β–carotene is considered to be the most powerful physical quenching agent in foods and is a particularly effective quencher of singlet oxygen. Foote (1976) found that one molecule of β–carotene can quench 250 to1000 molecules of singlet oxygen at a rate of 1.3×10^{10} $M^{-1}s^{-1}$. Energy transfer from singlet oxygen to a quencher results in the formation of triplet oxygen and a triplet-state quencher: $^1O_2 + {}^1Q \rightarrow {}^3O_2 + {}^3Q$ (Beutner and others 2000). The triplet-state quencher may also be formed in the energy transfer reaction between singlet-state quencher and an excited triplet-state sensitizer, in what is referred to as a triplet sensitizer quenching reaction, thereby returning the sensitizer to ground state. Investigations into the ability of β–carotene to act as a quencher in food systems has been prominent in recent years.

The rate of singlet oxygen quenching by carotene is highly dependent on the number of conjugate double bonds in the carotenoid. Another important factor is the type and number of functional groups on the ring portion of the molecule. This becomes important in the solubility of carotenoid. Kobayashi and Sakamoto (1999) compared the quenching activity of β-carotene to astaxanthin and found that the quenching activity of astaxanthin decreased with increasing hydrophobicity while the quenching activity of. β-carotene increased. Lee and Min (1990) evaluated the effectiveness of five carotenoids in quenching chlorophyll-sensitized photooxidation of soybean oil and reported that the effectiveness increased with the number of double bonds in the carotenoid and the amount of carotenoid added. Those carotenoids with seven or less double bonds are ineffective as quenchers, being unable to accept the energy from singlet oxygen. A comparison of quenching rates of several polyenes and carotenoids was reported Beutner and others (2000). β–carotene is one of best natural quenchers to used to minimize singlet oxygen oxidation of foods.

Tocopherols

Tocopherols are well-studied free radical scavengers, are the most abundant antioxidants in nature and are the primary antioxidant in vegetable oils. When present in systems that are vulnerable to singlet oxygen oxidation, tocopherols have demonstrated the ability to inhibit lipid peroxidation. Tocopherols were identified in soybean oil averaging about 1100 ppm and exist in α-, β-, γ- and δ-tocopherol forms at approximately 4, 1, 67 and 29% respectively (Jung and others 1991). Jung and others (1991) studied the effectiveness of α-, γ- and δ-tocopherol in quenching the photooxidized singlet oxygen

oxidation of soybean oil and determined that α -tocopherol had the greatest antioxidant effect. Foote and others (1970) found that the mechanism by which tocopherols act in quenching singlet oxygen involves charge transfer. The reaction involves an electron donation from tocopherol to singlet oxygen forming a charge transfer complex. The transfer complex undergoes an intersystem crossing to ultimately form triplet oxygen and the starting tocopherol. The overall rate is dependant on the ability of the donor to accept electrons in that the most easily oxidized donors are the most efficient quenchers.

Singlet oxygen loss of vitamin D_2 in a model system was reduced by the presence of α–tocopherol proportional to its concentration in solution (King and Min 1998). The rate of singlet oxygen quenching by α–tocopherol is similar to that of β–carotene.

Determining Quenching Mechanisms

The quenching mechanism of photosensitized singlet oxygen oxidation can be determined by measuring the rate constant of total quenching, physical quenching and chemical quenching. Quenching agents work in numerous ways to inhibit the formation of oxidized products, as has been previously described (Figure 2.20).

The quantum yield of a photochemical reaction is defined as the ratio of the number of molecules of a product formed to the number of photons of light absorbed. This value is used to measure the relative efficiency of a photochemical reaction. The quantum yield of oxidized product formation ($ØAO_2$) can be defined by the equation:

$$\mathbf{ØAO_2 = A \times B \times C} \qquad \mathbf{(1)}$$

where A and B represent the partitioning of singlet sensitizer and triplet sensitizer for singlet oxygen formation, respectively, and C represents the formation of the oxidized product.

The amount of quencher necessary to inhibit a substantial amount of the singlet oxygen sensitizer is particularly high and the lifetime of the singlet oxygen sensitizer is very short. For these reasons, the singlet sensitizer quenching is not considered in the steady state equation. Therefore, A is a constant (K) that is equal to the quantum yield of intersystem crossing.

Term B represents the rate of singlet oxygen formation, which is dependant on the triplet sensitizer quenching rate and the rate of triplet-triplet sensitizer annihilation. Therefore:

$$B = \frac{k_o\,[\text{oxygen}]}{k_o\,[\text{oxygen}] + k_Q\,[\text{quencher}]} \qquad (2)$$

where k_o is the reaction rate constant of triplet-triplet annihilation and k_Q is the reaction rate constant of triplet sensitizer quenching.

Term C represents the formation of oxidized product, which is dependant on the concentration and nature of the compound, physical and chemical quenching of singlet oxygen as well as the natural decay rate of singlet oxygen. The assemblage of these factors generates the following equation:

$$C = \frac{k_r\,[\text{compound}]}{k_r\,[\text{compound}] + (k_{ox\text{-}Q} + k_q)\,[\text{chemical + physical quencher}] + k_d} \qquad (3)$$

where k_r is the reaction rate constant of the reaction of singlet oxygen with the compound, $k_{ox\text{-}Q}$ is the reaction rate constant of chemical quenching, k_q is the reaction rate constant of physical quenching and k_d is the decay constant of singlet oxygen.

If a given quenching agent were to inhibit photosensitized oxidation by quenching singlet oxygen, the steady state equation can be written as:

$$\text{ØAO} = K \frac{k_o [^3O_2]}{k_o [^3O_2] + k_Q [Q]} X \frac{k_r [A]}{k_r [A] + (k_{ox\text{-}Q} + k_q) [Q] + k_d} \quad \textbf{(4)}$$

where K is the quantum yield of intersystem crossing of the excited state of the singlet sensitizer (term A from equation 1) and both B and C have been appropriately substituted with equations 2 and 3 respectively.

In a given system, if there is only singlet oxygen quenching such that $k_Q[Q] << k_o[^3O_2]$, then the B term is equal to 1. Therefore, the steady state equation becomes:

$$\text{ØAO} = K \frac{k_r [A]}{k_r [A] + (k_{ox\text{-}Q} + k_q) [Q] + k_d} \quad \textbf{(5)}$$

This equation can be inverted to:

$$\text{ØAO}^{-1} = K^{-1} \left(1 + \frac{(k_{ox\text{-}Q} + k_q) [Q] + k_d}{k_r} [A]^{-1} \right) \quad \textbf{(6)}$$

so that it is in slope-intercept form.

Alternatively, if there is only triplet sensitizer quenching such that $(k_{ox\text{-}Q} + k_q)[Q] << k_r[A] + k_d$, then the slope intercept form of the equation is:

$$\text{ØAO}^{-1} = K^{-1} \left(1 + \frac{k_Q[Q]}{k_o [^3O_2]} \right) \left(1 + \frac{k_d}{k_r [A]} \right) \quad \textbf{(7)}$$

The significance of these two equations is the fact that one describes a system in which singlet oxygen quenching is dominant and the other describes a system in which triplet sensitizer is dominant. A plot of $[AO_2]^{-1}$ vs. $[A]^{-1}$ at different [Q] will appear in one of two manners, dependant on which mechanism dominates a system. If singlet oxygen quenching is dominant (eq. 6), then the plots at various [Q] will all have the same y-intercept, but different slopes (Figure 2.22). If triplet sensitizer quenching is dominant (eq. 7), then both the intercept and the slope will vary (Figure 2.23).

	$^3O_2$	$^1O_2$
Energy Level	0	22.5 Kcal/mole
Nature	Diradical	Non-Radical, Electrophillic
Reaction	Radical Compound	Electron Rich Compounds

Table 2.1. Comparison of singlet and triplet oxygen (Min 2000)

	Oleate	Linoleate	Linolenate
Singlet Oxygen	9-OOH 10-OOH		
Conjugated Hydroperoxides		9-OOH 13-OOH	9-OOH 12-OOH 13-OOH 16-OOH
Nonconjugated Hydroperoxides		10-OOH 12-OOH	10-OOH 15-OOH

	Oleate	Linoleate	Linolenate
Triplet Oxygen	8-OOH 9-OOH 10-OOH 11-OOH		
Conjugated Hydroperoxides		9-OOH 13-OOH	9-OOH 12-OOH 13-OOH 16-OOH

Table 2.2. Hydroperoxides formed by singlet and triplet oxygen oxidation of fatty acids. Singlet oxygen oxidation results in the formaition of only 2 hydroperoxides from oleate. However, both conjugate and nonconjugate hydroperoxides are formed from linoleate and linolenate. (Frankel and others 1979)

	C18:1	C18:2	C18:3
Triplet Oxygen	1	27	77
Singlet Oxygen	3×10^4	4×10^4	7×10^4

Table 2.3. Relative oxidation rates of triplet and singlet oxygen with oleate, linoleate and linolenate (Gunston 1986).

	Added Chlorophyll		
Light Exposure (days)	0 ppm	1 ppm	5 ppm
1	0	0	0
2	0	0	0
3	0	0	1502
4	0	1534	3018

* Electronic counts of gas chromatogram

Table 2.4. 2-pentenyl furan peak areas in soybean oil (Callison 2001).

	Ascorbic Acid Exposure Time (hours)			Riboflavin Exposure Time (hours)		
Packaging Materials	1	2	3	1	2	3
Unexposed	0	0	0	0	0	0
Carton	5.5	35.0	35.0	3.2	3.7	9.1
Brown Bottle	18.0	66.0	71.0	7.5	7.5	11.0
Clear Bottle	89.0	92.0	96.0	28.0	39.0	50.0
White Sachet	71.0	94.0	96.0	25.0	36.0	50.0

Table 2.5. Destruction of ascorbic acid and riboflavin in milk by sunlight (expressed as % loss). (Satter and deMan 1975)

Quenching Compound	Quenching Rate
β-Apo-8'-carotenal	3.1×10^9 $M^{-1}sec^{-1}$
β-Carotene	4.6×10^9 $M^{-1}sec^{-1}$
Lutein	5.7×10^9 $M^{-1}sec^{-1}$
Zeaxanthin	6.8×10^9 $M^{-1}sec^{-1}$
Lycopene	6.9×10^9 $M^{-1}sec^{-1}$
Isozeqaxanthin	7.4×10^9 $M^{-1}sec^{-1}$
Astaxanthin	9.9×10^9 $M^{-1}sec^{-1}$
Canthaxanthin	11.2×10^9 $M^{-1}sec^{-1}$
α-Tocopherol	2.7×10^7 $M^{-1}sec^{-1}$
1,4-Diazabicyclo-(2,2,2)-octane	1.5×10^7 $M^{-1}sec^{-1}$
Dimethylfuran	2.6×10^7 $M^{-1}sec^{-1}$
BTC[1]	1.2×10^9 $M^{-1}sec^{-1}$
TPB[2]	3.7×10^7 $M^{-1}sec^{-1}$

[1] bis(di-n-butyldithiocarbamato)nickel chelate

[2] {2,2'-thiobis(4-1,1,3,3,-tetramethylbutyl)phenalto)]-n-butylamine)nickel chelate

Table 2.6. Singlet oxygen quenchers and their quenching rates. (Min and Lee 1999)

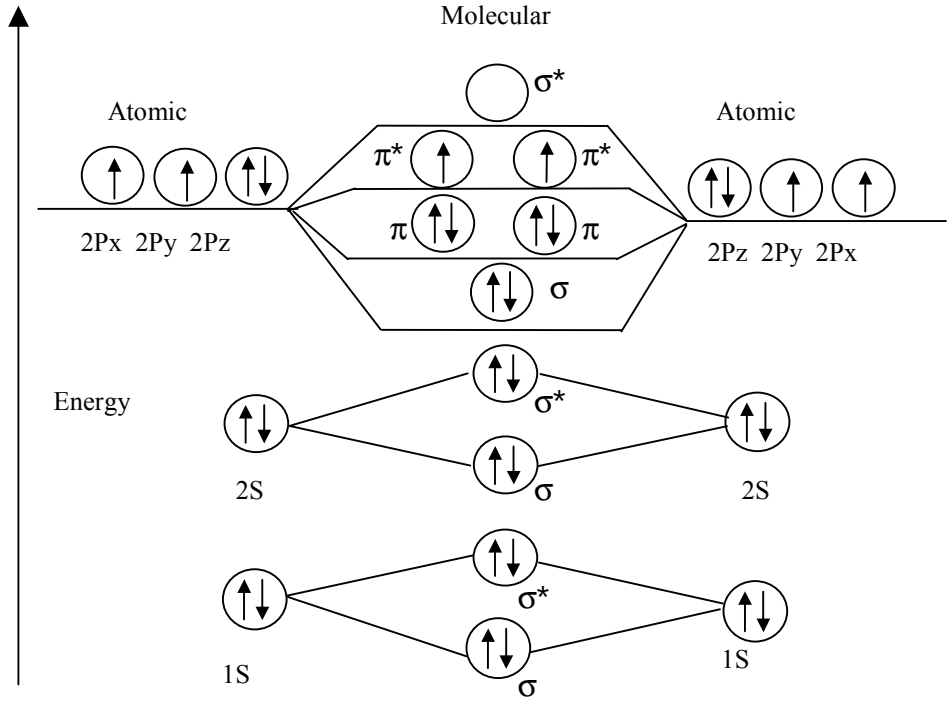


Figure 2.1. Molecular orbital of triplet oxygen (Min and Lee 1999)

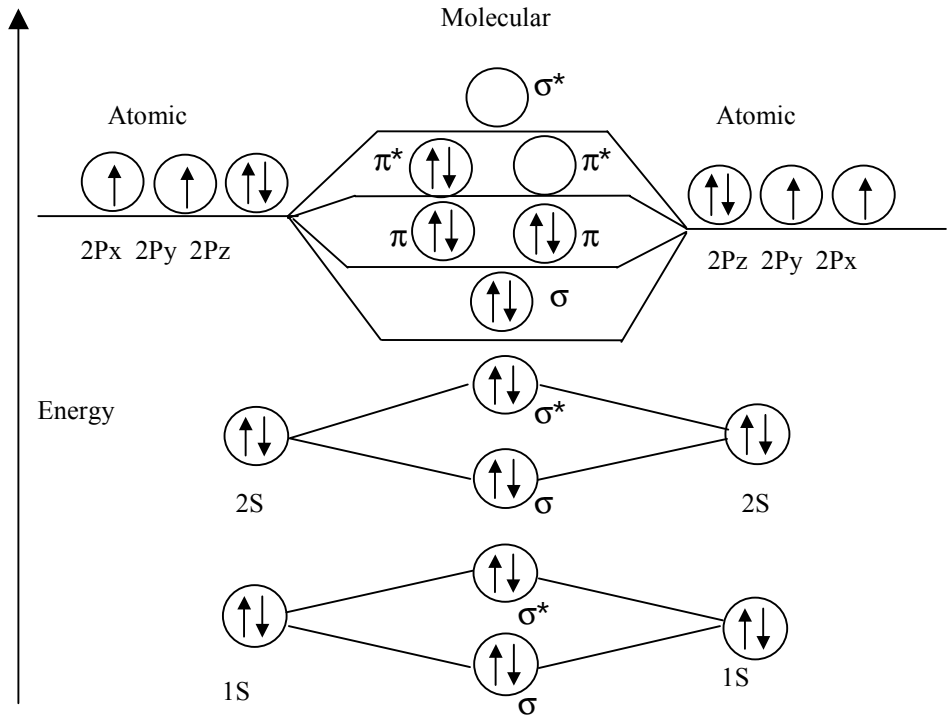


Figure 2.2. Molecular orbital of singlet oxygen (Min and Lee 1999)

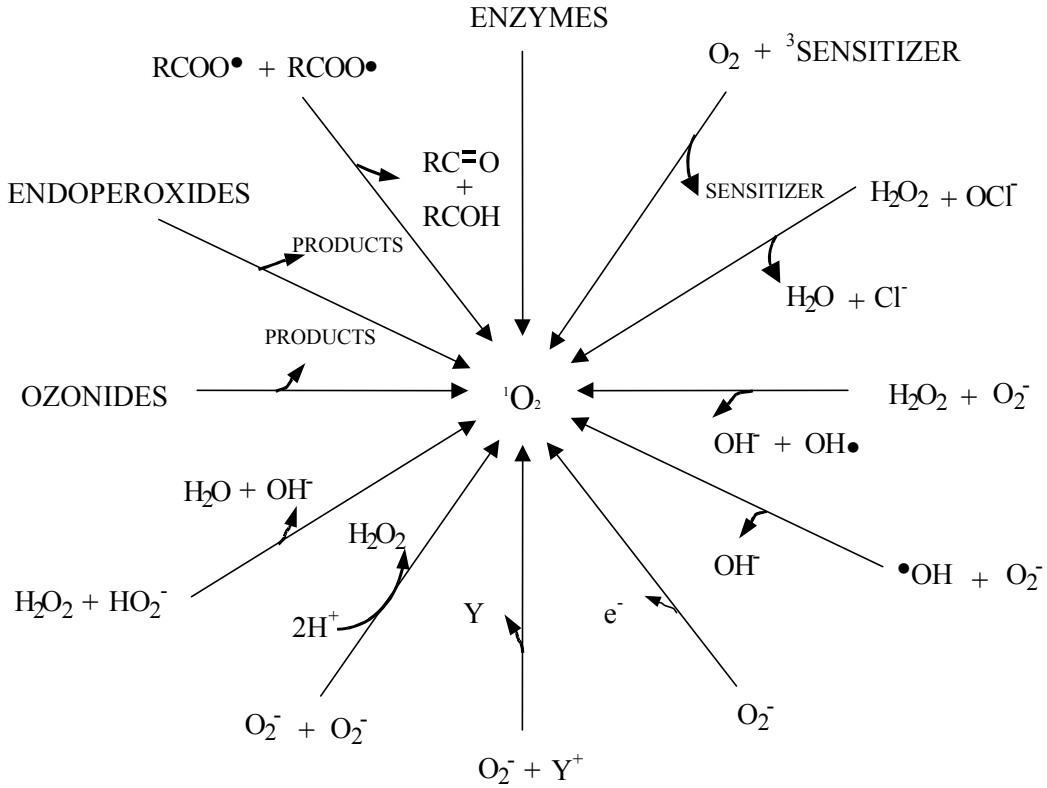


Figure 2.3. Singlet oxygen formation by chemical, photochemical and biological methods (Krinsky 1977)

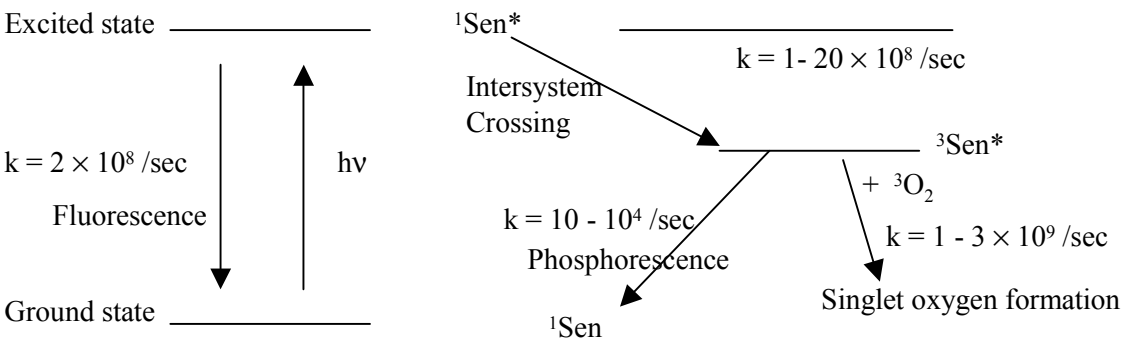


Figure 2.4. The chemical mechanism for the formation of singlet oxygen in the presence of sensitizer, light and triplet oxygen showing the excitation and deactivation of photosensitizer in the formation of singlet oxygen.

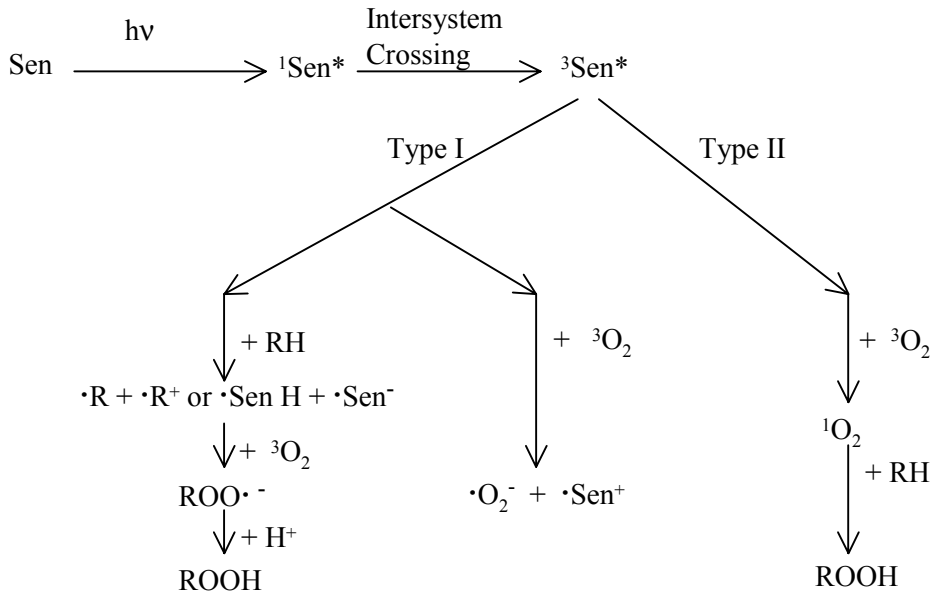


Figure 2.5. Formation of excited triplet sensitizer (^{3}Sen*) and its reaction with substrate via Type I and Type II reactions (Sharman and others 2000)

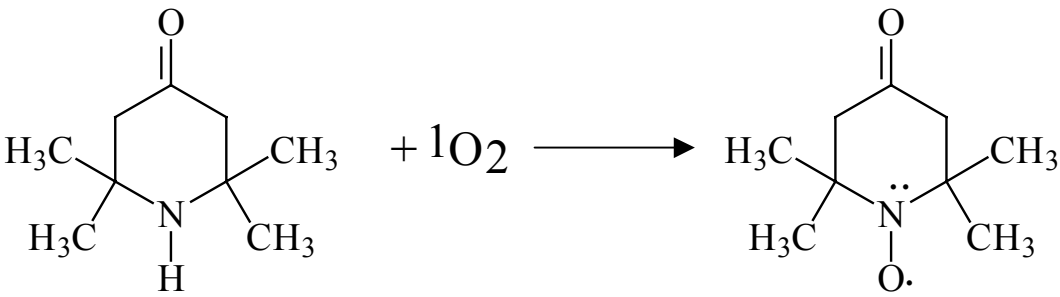


Figure 2.6. Reaction of 2,2,6,6-tetramethyl-4- piperidone with singlet oxygen for 2,2,6,6-4-piperidone-N-oxyl formation (Bradley 1991)

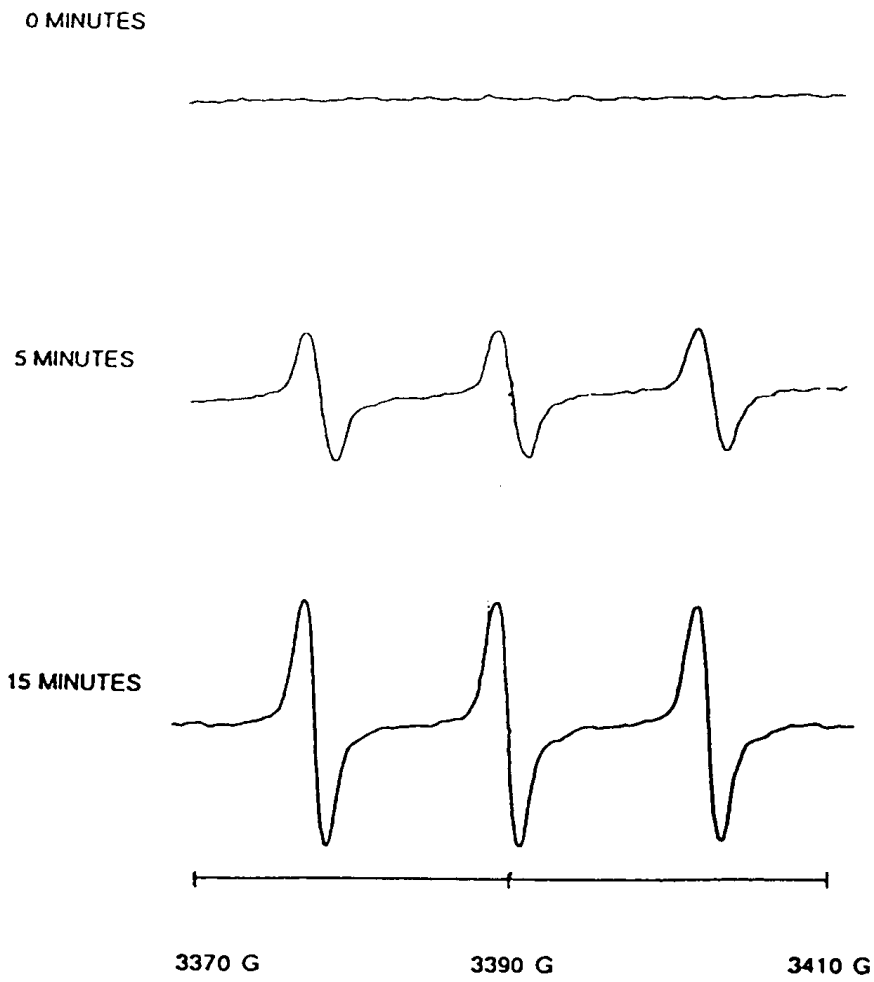


Figure 2.7. Effects of 0, 5 and 15 minutes illumination on electron spin resonance spectrum of 2,2,6,6-4-piperidone-N-oxyl in water solution of riboflavin and 2,2,6,6-tetramethyl-4- piperidone (Min and Lee 1996)

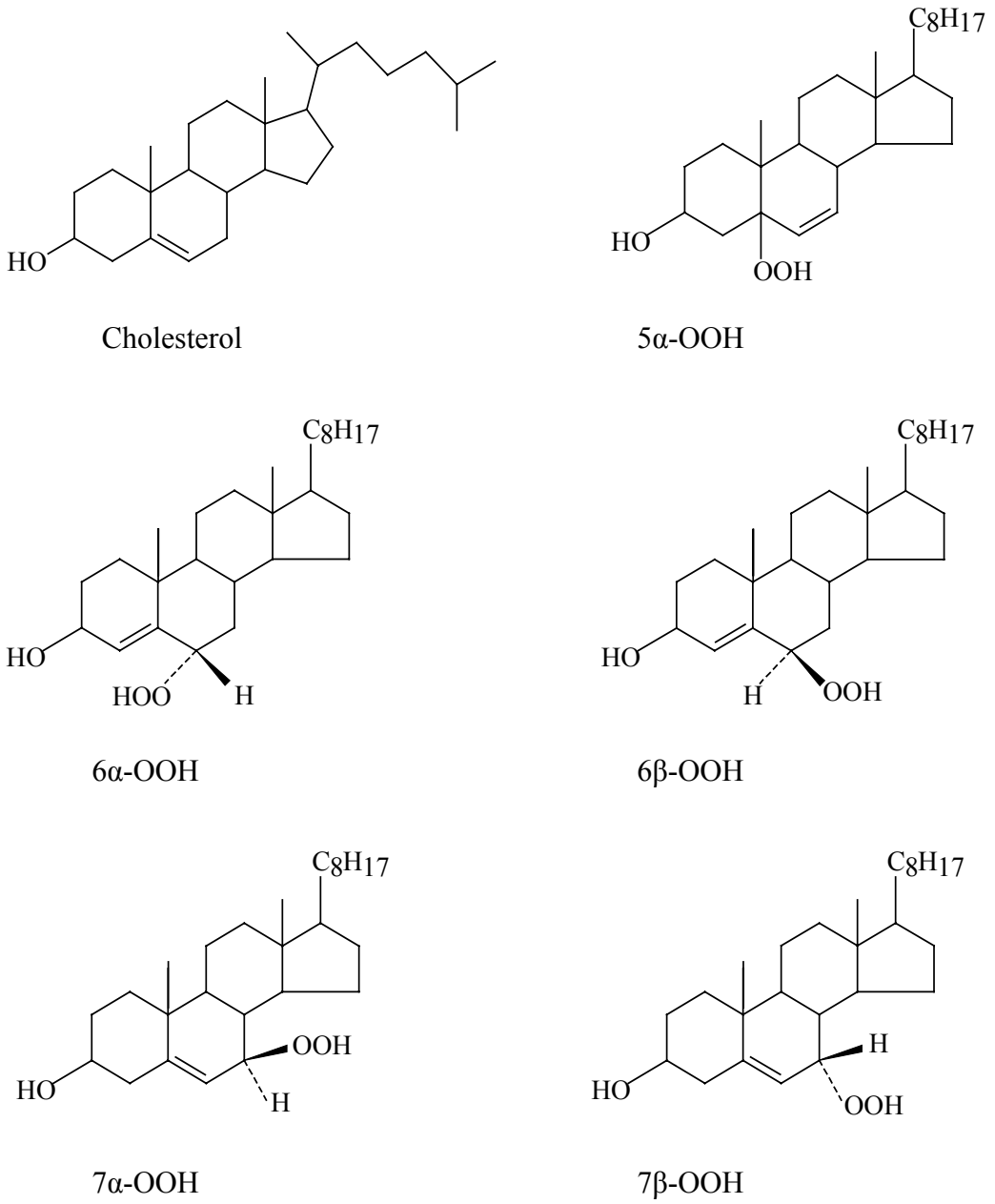


Figure 2.8. Cholesterol, its singlet oxygen oxidation products: 3β-hydroxy-5α-cholest-6-ene-5-hydroperoxide (5α-OOH), 3β-hydroxycholest-4-ene-6α-hydroperoxide (6α-OOH), and 3β-hydroxycholest-4-ene-6β -hydroperoxide (6β -OOH) and triplet oxygen oxidation products3β-hydroxycholest-5-ene-7α -hydroperoxide (7α -OOH) and 3β-hydroxycholest-5-ene-7β -hydroperoxide (7β -OOH). (Girotti 1998)

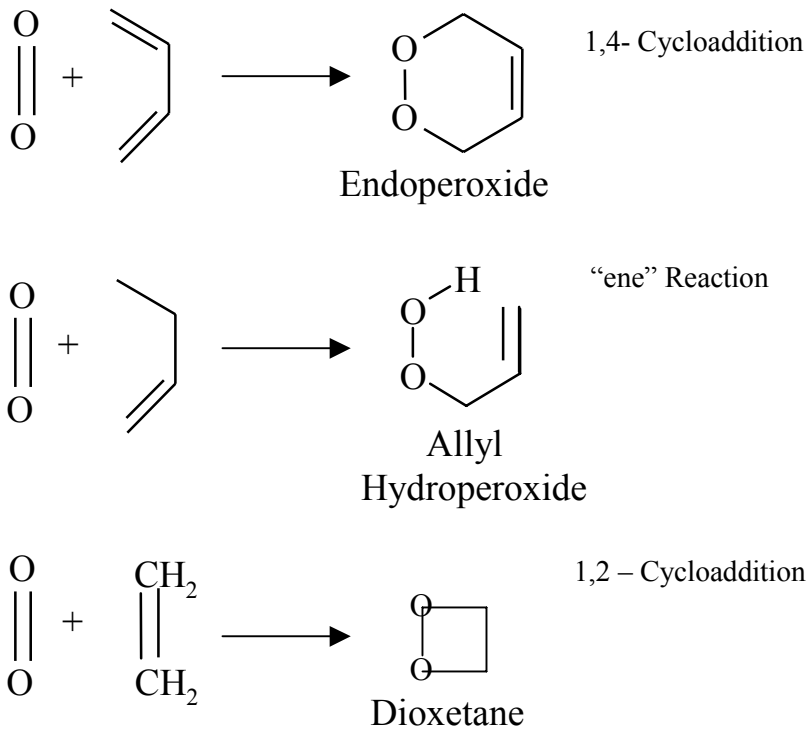


Figure 2.9. Reactions of singlet oxygen with olefins by: 1,4-cycloaddition, the "ene" reaction, and 1,2-cycloaddtion (King 1996)

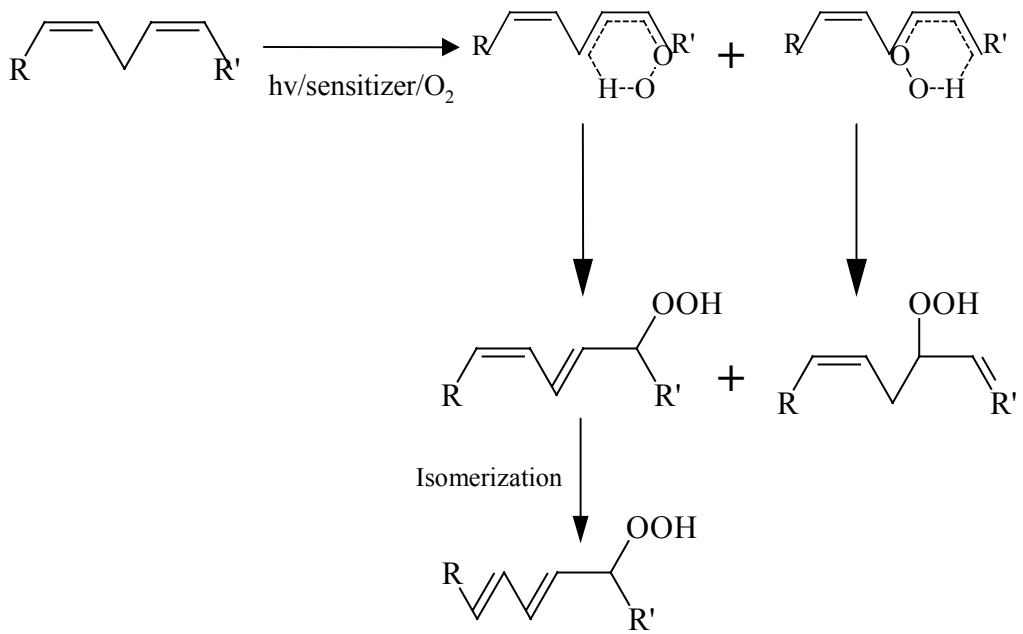


Figure 2.10. Conjugated and nonconjugated hydroperoxide formation from a diene fatty acid by the "ene" reaction of singlet oxygen. Two hydroperoxide intermediates are formed (Min and Lee 1999)

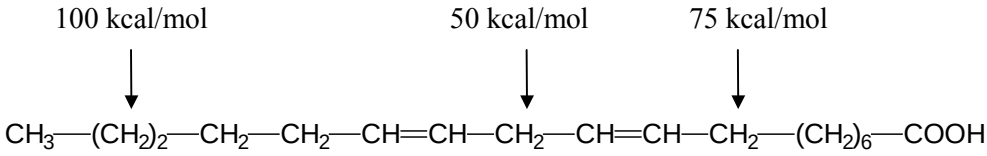


Figure 2.11. Energy required for hydrogen removal from linoleic acid. Hydrogen at position 11 of linoleic acid is most easily removed due to the presence of a double bond on both sides. (Frankel 1985)

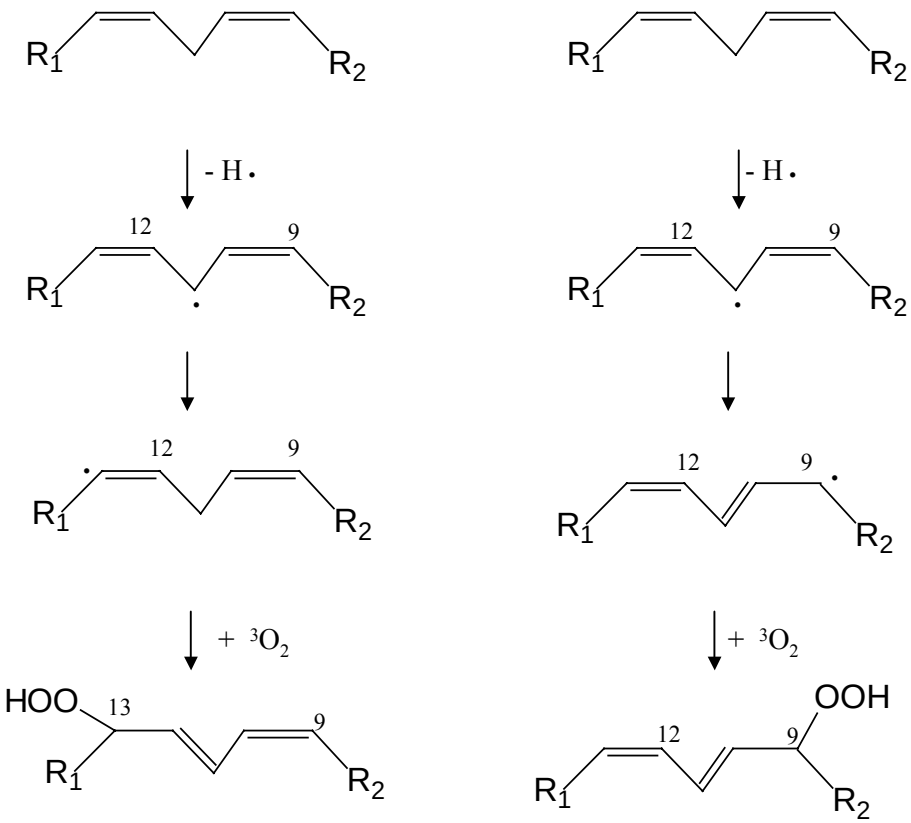


Figure 2.12. Conjugated hydroperoxide formation from linoleic acid by free radical reaction of triplet oxygen. An equal mixture of position 9 and 13 hydroperoxides are produced. (Min and Lee 1999)

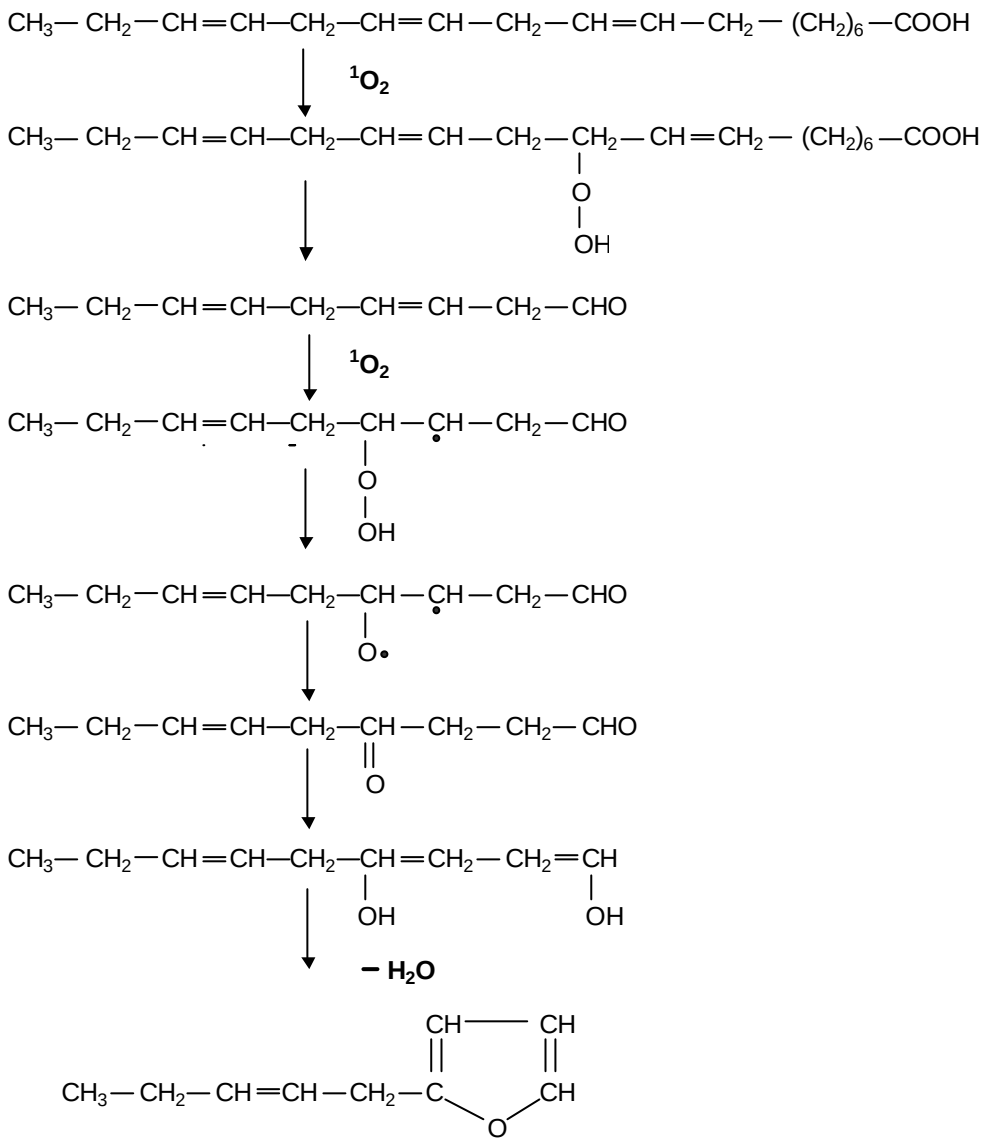


Figure 2.13. Mechanism for the formation of 2-pentenyl furan from linolenic acid by singlet oxygen (Min 2000)

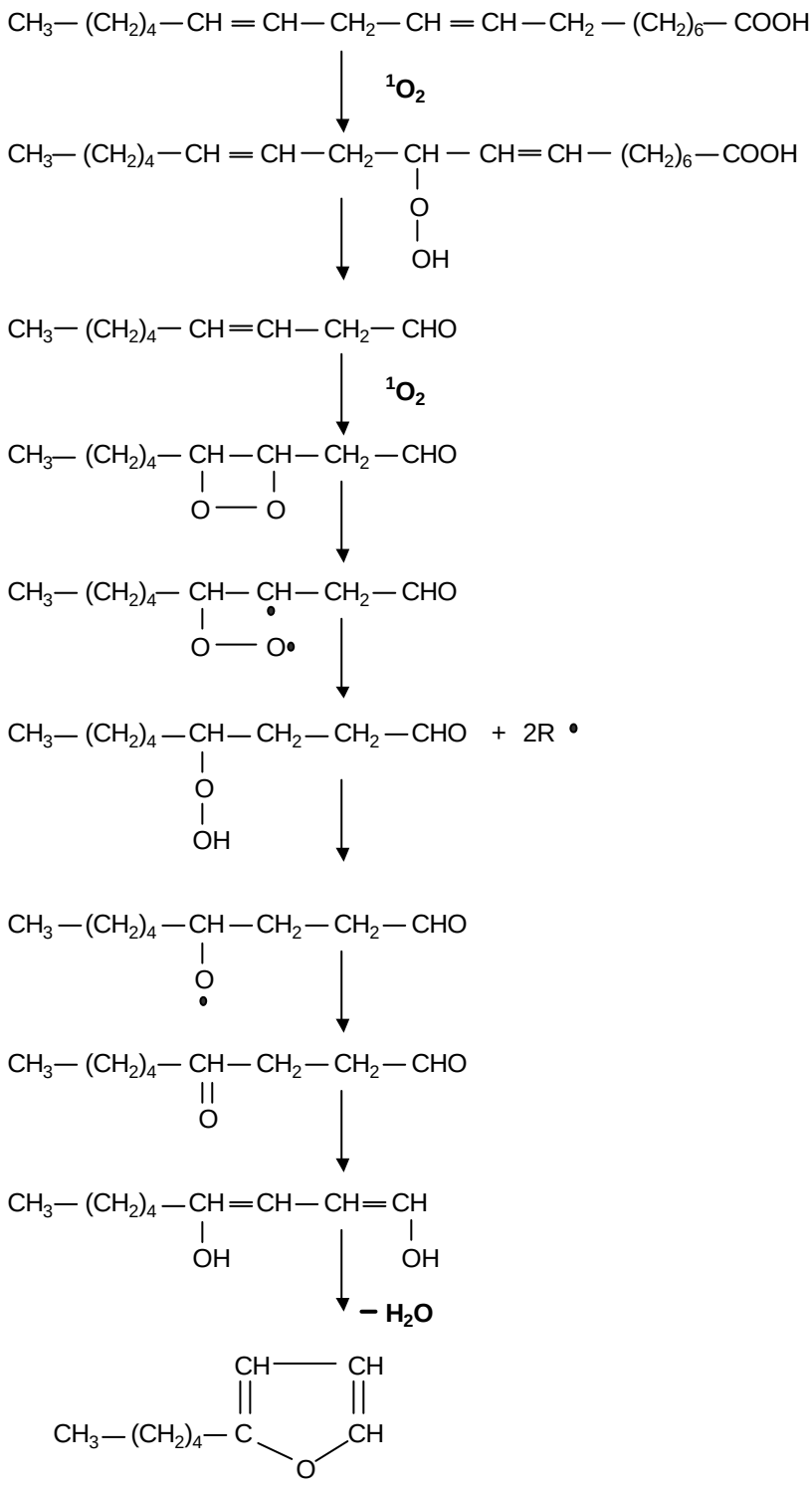


Figure 2.14. Mechanism for the formation of 2-pentyl furan from linoleic acid by singlet oxygen (Min 2000)

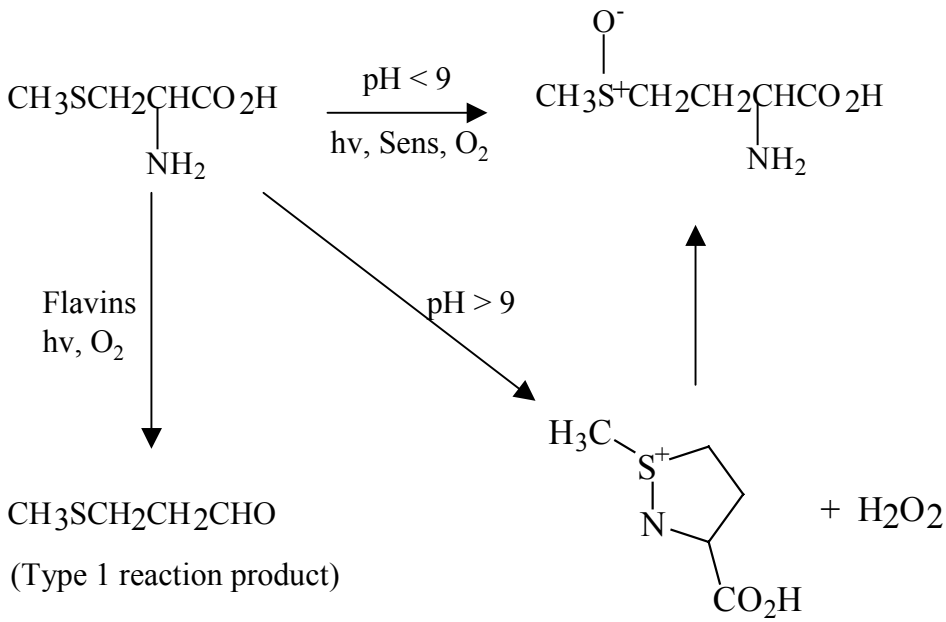


Figure 2.15. Reactions of methionine with singlet oxygen (Foote 1976)

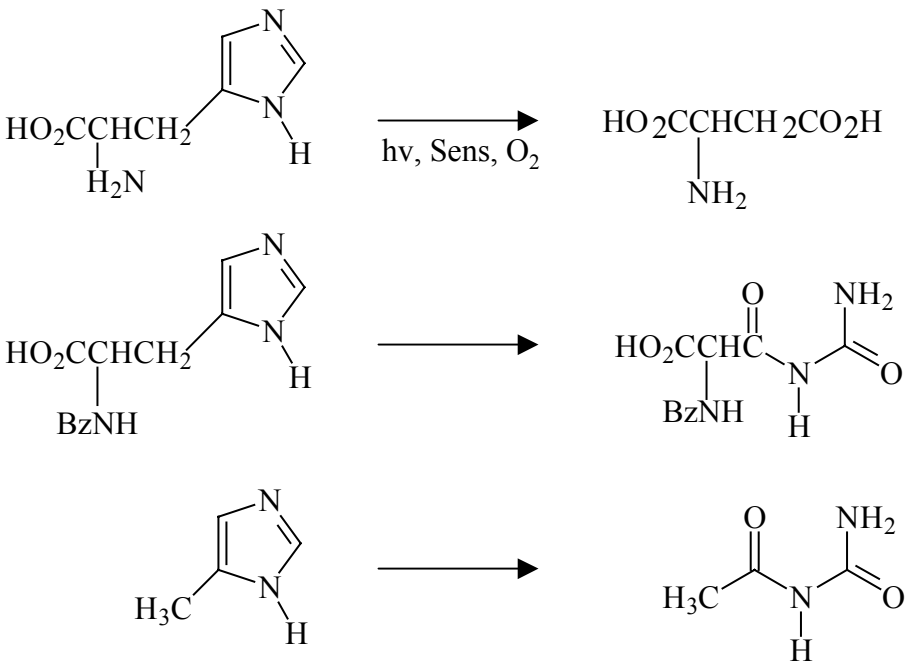


Figure 2.16. Reactions of histadine with singlet oxygen (Foote 1976)

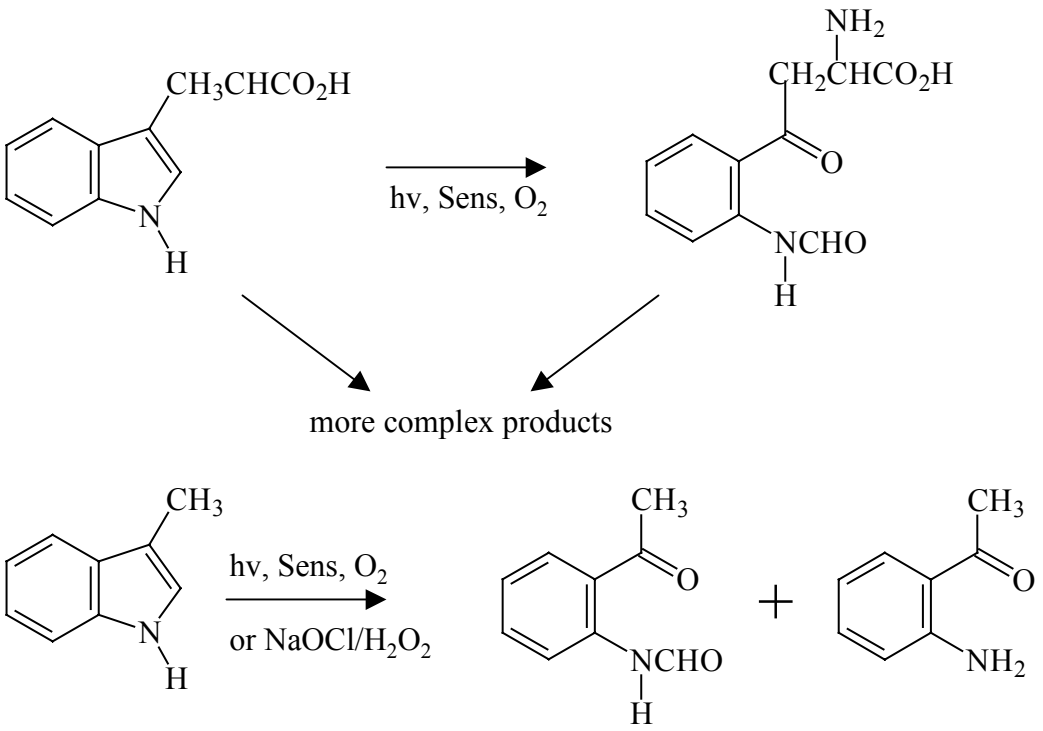


Figure 2.17. Reactions of tyrosine with singlet oxygen (Foote 1976)

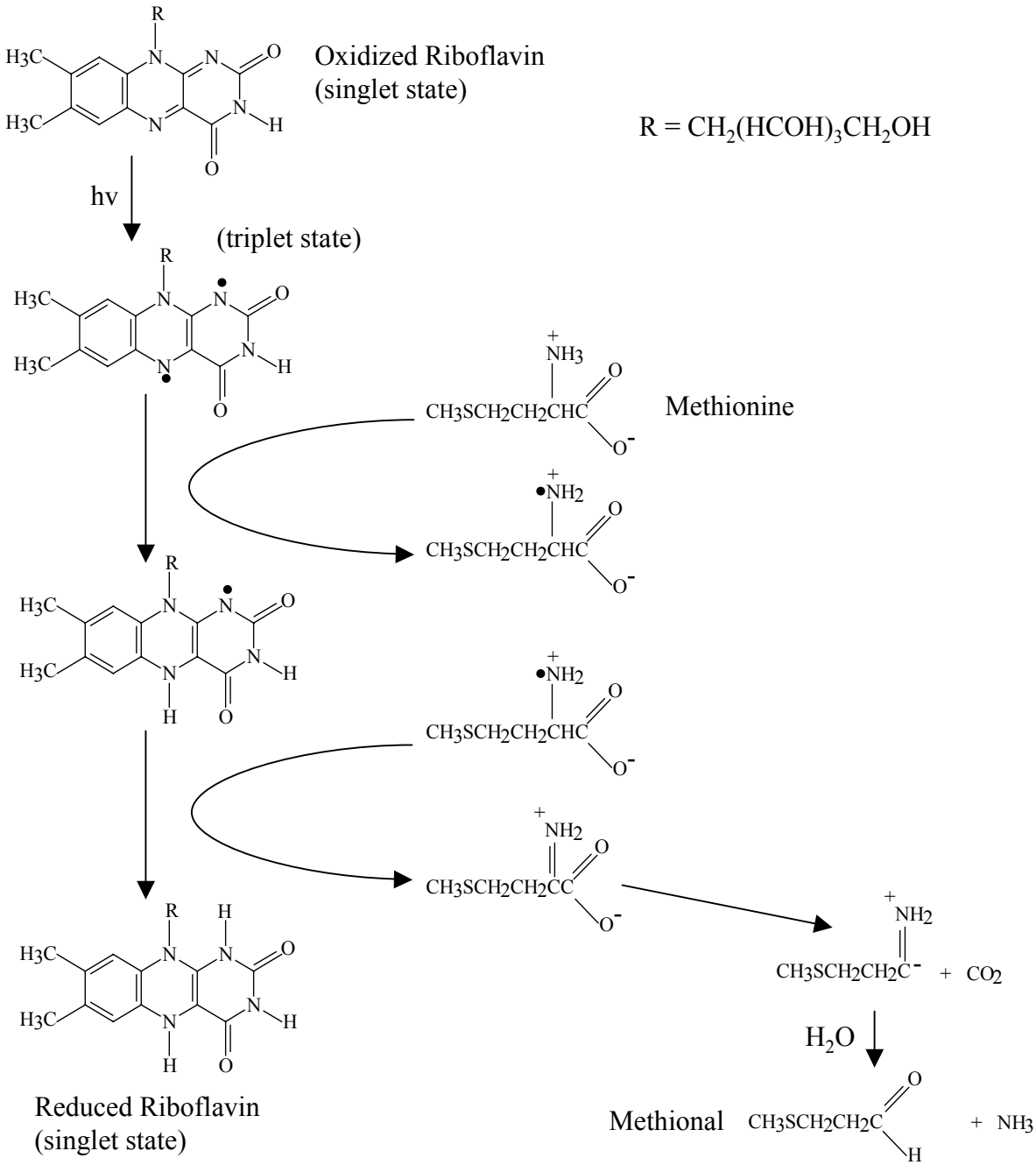


Figure 2.18. Proposed riboflavin catalyzed Strecker degradation-like reaction of methionine to form methional (Hoskin 1979)

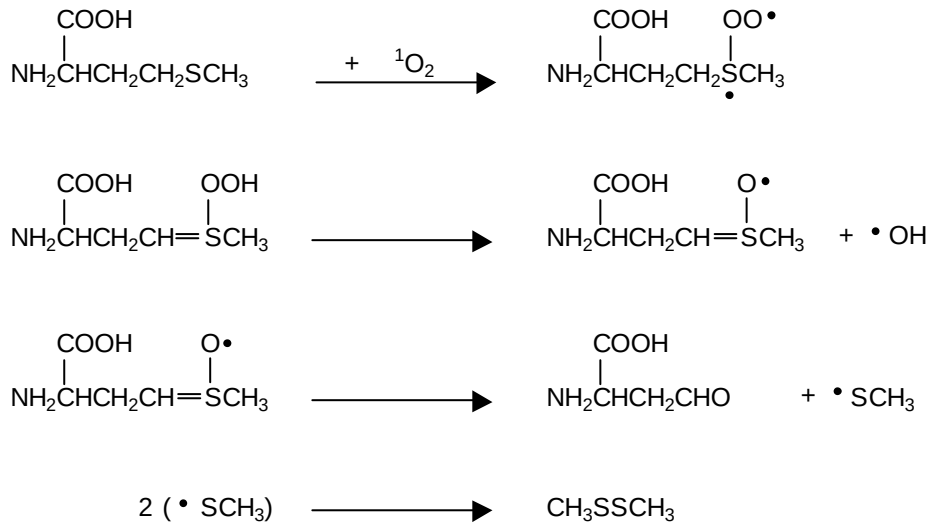


Figure 2.19. Formation of dimethyl disulfide from methionine and singlet oxygen (Jung and others 1998)

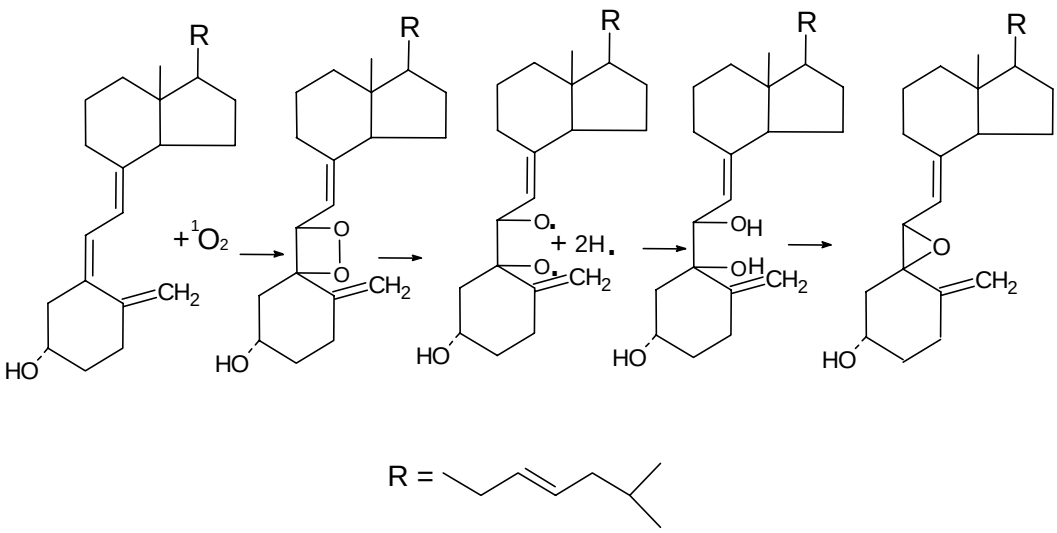


Figure 2.20. Proposed chemical mechanism for the formation of 5,6 –epoxide from vitamin D_2 by singlet oxygen (King 1996)

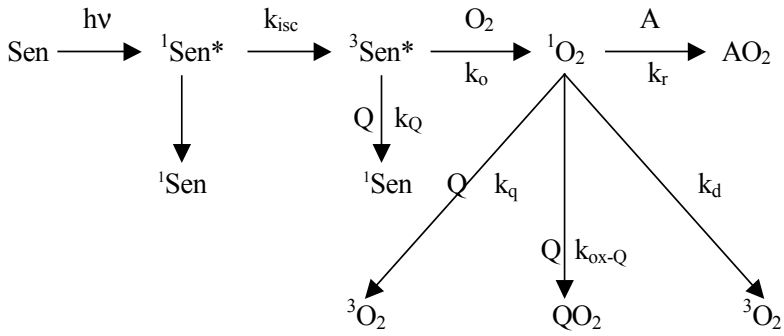


Figure 2.21. Formation of singlet oxygen and its reaction with substrate A to produce the oxidized product AO_2. The formation of AO_2 can be prevented by the reaction of ^{3}Sen* or $^1O_2$ with a quenching agent. (Min and others 1989)

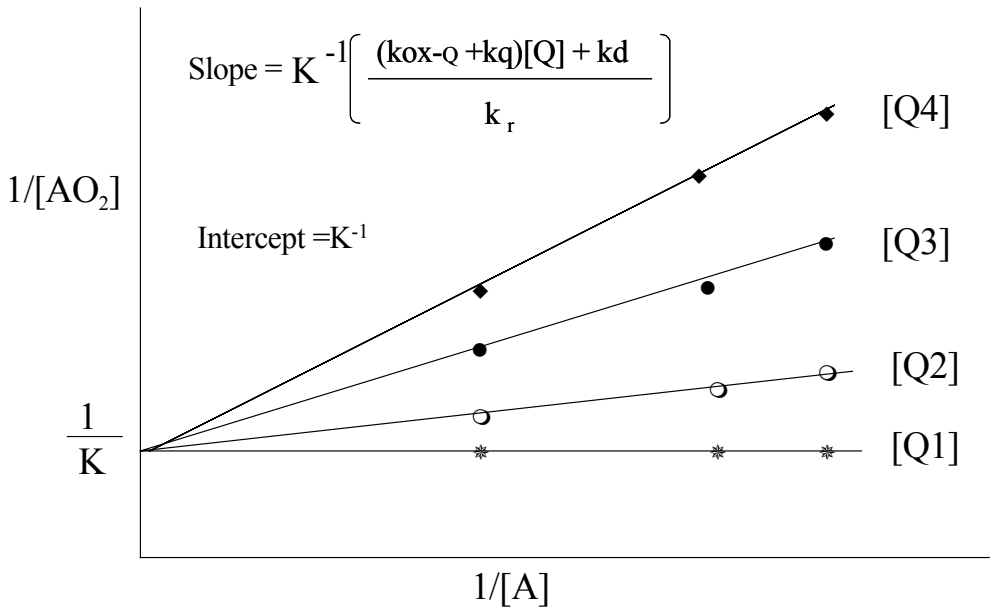


Figure 2.22. Characteristic plot of a singlet oxygen quenching mechanism (Li and others 2000)

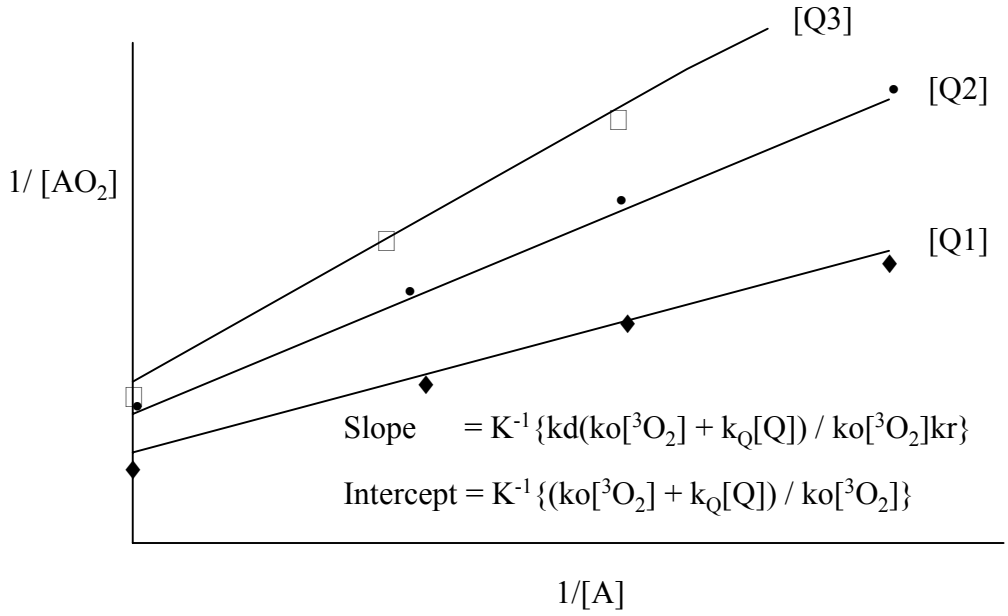


Figure 2.23. Characteristic plot of a triplet sensitizer quenching mechanism (Li and others 2000)

2.2. Solid Phase Microextraction Theory and Application

Solid phase microextraction (SPME) is a method used to collect chemical constituents from a sample. SPME can be use for direct sampling or headspace sampling. Direct sampling requires the insertion of the solid phase fiber into the sample. Headspace sampling involves insertion of the solid phase fiber into the headspace of a sealed vial containing the sample. Direct sampling is more sensitive; however, it is often detrimental or destructive to the quality of the fiber. Direct sampling is commonly used for non-volatile compounds and easily desorbed compounds. Headspace sampling is commonly used for collection of volatile compounds.

The use of SPME is beneficial due to being both faster and less complex than extraction/purification methods. The result is that fewer opportunities for errors are present and sample extractions can be performed quickly, with excellent reproducibility and at a lower cost compared to extraction/purification methods.

The fused silica fiber is coated with a stationary phase that has affinity to the constituents of a sample product. The affinity of the sample constituents to the fiber is primarily due to the polarity of both the chemicals and the stationary phase of the fiber coating. A commonly used polar coating if poly acrylate (PA), and a commonly used nonpolar coating is poly(dimethylsiloxane) (PDMS). A sample product is placed in a sealed vial and a fiber coated with the solid phase material is inserted into the headspace of the vial. Volatile chemicals will migrate from the aqueous or solid food into the headspace and then from the headspace to the fiber. Therefore, collection of volatiles by headspace SPME is dependant on the equilibrium between the concentration of the volatile compound in the solid phase, the sample headspace, and the sample. The

volatiles collected on the fiber are then desorbed into a gas chromatograph for analysis. The following equation describes the equilibriums involved in headspace volatile extraction:

$$n = \frac{K_{fs}V_fV_sCo}{K_{fs}V_s+K_{fh}V_h+V_s}$$

Where n = the collected volatile, Co is the initial concentration of the volatile in sample, K_{fs} is the partition between the fiber and the aqueous phase, K_{fh} is partition between the fiber and the headspace and V_f, V_s, V_h are the volumes of the fiber material, aqueous (sample) and headspace, respectively.

Since the equilibrium of different compounds in these three phases can be affected by several factors, SPME may not be an ideal method for all types of products. Careful selection of solid phase material, sample preparation, extraction conditions and analytical conditions can improve results obtained from SPME-GC-MS analysis of volatile compounds. Almost all of the published research on the use of SPME in volatile analysis of foods has been within the past five years; therefore, information regarding the use of SPME for the collection of volatile compounds in foods is still rapidly developing.

The SPME analysis of volatiles is influenced by several factors. One of the most important of which is the collection of volatile chemicals on the fiber. As previously stated, collection on the fiber is dependant on the ability of the volatiles to get from the food material to the fiber. The selection of the appropriate fiber must be made so that the volatiles have affinity for the fiber.

The larger the initial volume of the sample, the more volatiles present and the more likely it will be to obtain enough compound for analysis. Other things can be done

to increase the amount of volatiles extracted. Stirring increases the diffusion rate of compounds into the sample headspace, thereby reducing the limiting rate of diffusion.

Increasing the extraction temperature generally results in an increase in the amount of volatile compounds that will be extracted. However, excessive temperatures could accelerate chemical reactions, potentially altering the sample and the information that can be gathered from it. Extraction time also is also a consideration. Short extraction time is ideal, minimizing the acceleration in chemical reactions that may occur at the extraction temperature. Reproducibility in measurement is a matter of consistency in sampling/extraction procedures.

2.3. Orange Juice

Orange Juice Flavor

Orange juice is the most popular fruit juice in the U.S. market today. Over 94% of the Florida orange juice crop used for orange juice production (Baker and Cameron 1999; Moshonas and Shaw 2000). The chemical and physical properties of orange juice can vary among species, region, environment and climate. However, oranges and orange juice can be generally described by certain important constituents. The flavor of orange juice is predominantly derived from its volatile compounds, which are heat-labile (Steffan and Pawliszyn 1996). Numerous studies have identified and assessed the contribution of various volatile compounds to the flavor and aroma of orange juice (Shaw and others 1993; Moshonas and Shaw 2000; Farnworth and others 2001). Qualitative and quantitative analysis of volatiles may be used to assess the flavor quality of orange juice (Moshonas and Shaw 2000). Important flavor chemicals are predominantly hydrocarbons (75-98%). Terpenic hydrocarbons such as α-pinene, α-phellandrene, limonene, caryophyllene and valencene are important flavor constituents and are abundant components of orange peel oil (Jordan and others 2001). Aldehydes comprise 0.6 to 1.7% with esters and ketones comprising about 1% each. Decanal is considered an important contributor to the aroma of orange juice (Jordan and others 2001). Alcohols range from 1 to 5% of the total. The predominant chemical is limonene, but it is not the most important in terms of flavor quality as determined by sensory evaluation (Jia 1998). Limonene is also one of the most easily lost chemicals due to processing and storage; reports state that 40% can be lost during reasonably long storage periods, some of which degrades into α-terpineol, a known off-flavor of orange juice (Jordan and others 2001).

Orange Juice Processing

Orange juice is heat pasteurized to inactivate pectinmethylesterase (PME) and to inactivate spoilage organisms. Wicker and Temelli (1988) reported that 96% of the PME is inactivated after 180 seconds at 80 ^{o}C. The lack of complete inactivation is due to the thermal-stable PME fraction. Orange PME is bound to the cell walls and is released upon extraction (Rombouts and others 1982; Van den Broeck and others 1999). PME hydrolyses the C-6 methyl esters of polygalacturonic acid residues in pectin to yield methanol and pectic acid (Cameron and others 1998). Divalent cations, such as calcium, can then cross-link pectic acid units to adjacent pectin chains, thereby reducing their solubility resulting in cloud loss (Wicker and Temelli 1988; Baker and Cameron 1999). Enzymatic clarification of the cloud in citrus juice is considered a product quality defect (Braddock 1999). Therefore, it is necessary to inactivate PME in order to produce an acceptable orange juice. However, the heating process degrades the organoleptic and nutritional qualities of the juice (Reynolds 1963, Klavons and others 1994, Braddock 1999).

High-pressure processing (HPP) can inactivate the heat-sensitive PME isozyme, and kill spoilage microorganisms, such as lactic acid bacteria, yeasts, and molds (Ogawa and others 1990; Basak and Ramaswamy 1998; Goodner and others 1998; Nienaber and Shellhammer 2001). High pressure in combination with high temperatures have been shown to be ineffective at completely inactivating PME activity in orange juice, leaving behind at least 10% residual PME activity after processing (Ogawa and others 1992; Basak and Ramaswamy 1998; Goodner and others 1998; Van den Broeck and others

1999; Nienaber and Shellhammer 2001). This suggests that the heat-stable PME isozyme is also pressure-stable. Balaban and others (1991) demonstrated that the use of supercritical CO_2 can inactivate PME and render a stable cloud in orange juice without the use of extremely high pressures.

Storage

During storage, volatile compound losses will occur in fresh and processed juice; such losses can be dependent on storage temperature, duration of storage and packaging (Ahrne and others 1996; Lee and Coates 1999; Moshonas and Shaw 2000; Yeom and others 2000). Moshomas and Shaw (2000) found that the most noticeable decrease in volatile constituents occurred at 4-5 weeks, with the water soluble constituents being more sensitive than the oil soluble. Water soluble constituents dropped to about 30% of their initial amounts and oil constituents dropped to about 70% of their initial amounts during 10 weeks storage at 2 ^{o}C. Limonene decreased to about 80% of its initial level after 2-9 weeks at 2 ^{o}C (Moshonas and Shaw 2000). Martin and others (1992) found that the amount of linalool increased due to thermal heating of the juice. This may be due to liberation of the linalool from its glucoside during heating.

CHAPTER 3

PHOTOXIDATION AND PHOTOSENSITIZED OXIDATION OF SOYBEAN OIL

3.1 Abstract

Lipid oxidation is an important quality concern of foods because lipids are highly susceptible to oxidation and products of lipid oxidation include low molecular weight volatile compounds that cause off-flavors in foods. Chlorophyll photosensitized oxidation can increase the rate of oxidation and development of volatile compounds from lipids resulting in product quality loss. The objectives of this research were to compare the effects of light exposure, chlorophyll concentration and storage temperature on the production of volatile compounds in soybean oil. Soybean oil containing 0, 1, 5 or 10 ppm chlorophyll was stored under light at 4, 20 and 40 °C for 7 days. Volatile compounds were analyzed by solid phase microextraction and gas chromatography and mass spectroscopy.

Light exposure had a significant effect on the production of all volatile compounds in soybean oil stored for 7 days at 4, 20 or 40 °C. Storage temperature was a significant factor to the production of all volatile compounds in soybean oil stored under

light. Chlorophyll concentration was a significant factor in the production of total volatiles and the singlet oxygen product 2-heptenal, but not on the triplet oxygen autoxidation product hexanal after 2 days of storage. The presence of 5 to 10 ppm chlorophyll in soybean oil significantly increased the production of approximately half of the volatile compounds in the soybean oil after.

3.2 Introduction

Oxidation of foods can negatively alter the flavor, color, nutrition and consumer acceptance of food products. These oxidative changes can result in economic losses to the food manufacturers and retailers. Products of oxidation include low molecular weight volatile compounds that cause noticeable off-flavors even at very low concentrations. The production of volatile compounds in edible oils is undesirable. Lipid oxidation is of particular concern to food manufacturers because lipids are highly susceptible to oxidation. For this reason, many lipid-containing foods are protected from oxidation through the addition of natural and synthetic antioxidants.

The oxidation of fatty acids in edible oils is dependant on several factors including storage time, temperature, the composition of the oil, and environmental conditions (Frankel 1985). Normal atmospheric triplet oxygen ($^3O_2$) readily reacts with radical compounds in foods (Frankel 1985). However, fatty acids are non-radical singlet state molecules. The fatty acid must first become a radical compound to react with the diradical triplet oxygen, initiating the oxidation reaction. Once initiated, the reaction can propagate a free-radical autoxidation. Fatty acid oxidation produces free-radicals that further propagate the oxidation reaction and ultimately produce undesirable volatile off-

flavor compounds (Steenson and Lee 2002). Autoxidation occurs more rapidly at elevated temperatures and in the presence of polyunsaturated fats, which are more susceptible to initiation than saturated and monounsaturated fatty acids.

Due to the importance of lipid oxidation to food quality and stability, triplet oxygen autoxidation has been extensively studied for the last 70 years. Within the past three decades increased attention has been given to another type of oxidation reaction know as singlet oxygen oxidation. The contribution of singlet oxygen ($^1O_2$) to oil oxidation can be quite significant because the rate of singlet oxygen oxidation is much greater than that of triplet oxygen autoxidation, and singlet oxygen oxidation mechanisms favor the formation of off-flavor volatile compounds that are not significantly formed by triplet oxygen autoxidation (Adam 1975; Beutner and others 2000). The rate of singlet oxygen oxidation is over 1450 times greater than triplet oxygen autoxidation (Rawls and VanSanten 1970; Farnkel and Neff 1981). Singlet oxygen can directly react with electron-rich double bonds without the initiation of free radical intermediates and rapidly increase the oxidation rate of foods even at very low temperatures (Min and others 1989). Products of singlet oxygen oxidation include volatile off-flavor compounds as well as reactive free-radical compounds. The free-radicals formed from singlet oxygen oxidation can initiate triplet oxygen autoxidation.

The most prominent mechanism for the formation of singlet oxygen in foods is by photosensitized generation. The photosensitizer absorbs ultraviolet or visible radiation energy and rapidly becomes an unstable, excited, singlet-state molecule ($^1Sen^*$). The excited singlet photosensitizer loses its energy by internal conversion, emission of light, or intersystem crossing as shown in Figure 3.1. Internal conversion involves the

transformation from high energy to a lower energy state by releasing energy as heat. Emission of fluorescent light results in the conversion of the ^{1}Sen* to ground state singlet sensitizer (^{1}Sen). The ^{1}Sen* may also undergo an intersystem crossing from the excited singlet-state molecule to an excited triplet-state molecule (^{3}Sen*). The ^{3}Sen* can react with $^{3}O_2$ to form $^{1}O_2$ and ^{1}Sen by a triplet-triplet annihilation mechanism. The sensitizer returns to ground state ^{1}Sen to repeat the cycle of generating singlet oxygen. Sensitizers may generate 10^3 to 10^5 molecules of singlet oxygen before becoming inactive (Min and others 1989; Kochevar and Redmond 2000). Many molecules can act as photosensitizers in foods; chlorophyll, riboflavin, myoglobin and some artificial colorants are known photosensitizers (Foote and Denny 1968; Afonso and others 1999; Lledias and Hansberg 2000). The presence of photosensitizers in foods is quite prevalent. Consequently, singlet oxygen oxidation in foods can be a major contributor to oxidation and the production of off-flavor compounds, which lowers consumer acceptability and nutritional quality.

Many edible oils derived from plants contain chlorophyll. Crude soybean oil contains 1-2 ppm of chlorophyll; while crude canola oil contains 5-25 ppm (Swern 1979; Brekke 1980; Koseoglu and Lusas 1990). This has been recognized by manufacturers, and refining methods to remove pigments adequately removes nearly all of the chlorophyll from edible oils. However, since edible oils are used in many products that can contain chlorophyll or other photosensitizers, photosensitized oxidation of edible oils can still be a major problem. Soybean oil is the most widely utilized edible oil in the United States due to availability and inexpensive cost. The amount of soybean oil produced in the United States accounts for approximately 70% of the total vegetable oil

produced, exceeding corn oil production by eight times (Golbitz 2000). It contains two polyunsaturated fatty acids, linoleic and linolenic acids, which are highly susceptible to autoxidation. For these reasons, soybean oil was selected for the evaluation of photosensitized oxidation by chlorophyll. The effect of chlorophyll on the formation of specific volatile compounds in soybean oil has not been thoroughly studied. It is well known that lipid autoxidation and the production of volatile compounds becomes more prominent with increasing temperatures. The objectives of this research were (1) to determine the effect of light exposure and chlorophyll concentration on the amount of total volatile compounds in soybean oil, (2) determine which volatile compounds in soybean oil are significantly affected by chlorophyll concentration and (3) compare the effect of light exposure, chlorophyll concentration and storage temperature on the amount of volatile compounds in soybean oil.

3.3 Materials and Methods

Materials

Refined, bleached and deodorized (RBD) soybean oil was obtained from Archer Daniels Midland Co. The RBD soybean oil contained less than 0.04 ppm residual chlorophyll. Chlorophyll b and the chemical standards 1–pentenol, hexanal, 2-hexenal, heptanal, 2-heptenal, 1-octen-3-ol and 2,4-heptadienal were purchased from Sigma Chemical Co. (St.Louis, MO). The chemical standard 2-pentyl furan was purchased from Karl Industries Inc. (Aurora, OH). The gas chromatography (GC) column, the polydimethylsiloxane/divinylbenzene (PDMS/DVB) solid phase microextraction fibers, fiber holder, glass GC inlet liners, 10ml glass serum vials, Teflon-coated rubber septa and

aluminum crimp caps were purchased from Supelco, Inc. (Bellefonte, PA). The chlorophyll b was stored at –20 °C until use. Other chemicals were stored at 4 °C.

Sample Preparation and Storage

Chlorophyll was added to the RBD soybean oil at concentrations of 1, 5, and 10 ppm and mixed with a stirring bar for 4 hours at room temperature in a sealed flask that had been flushed with nitrogen. A 2 ml aliquot of the prepared soybean oils containing 0, 1, 5 or 10 ppm added chlorophyll was placed in a 10 ml glass vial and sealed with a Teflon-coated rubber septa and aluminum crimp cap to prevent contamination of the vial headspace. The prepared vials of soybean oil were stored in a mirrored light box for 7 days while exposed to a tungsten light (1560 lumens). The temperature in the light box was maintained at 4, 20, or 40 °C (±2 °C) throughout the duration of the storage. A 2 ml aliquot of RDB soybean oil containing 0 ppm added chlorophyll was placed in a vial, sealed, wrapped with foil to prevent light exposure and stored in the light box for 7 days along with the other vials. Prior research has established that chlorophyll has no effect on oil quality if the oil is not exposed to light (Lee 2003); therefore, the oil stored in the dark did not contain added chlorophyll.

Analysis of Headspace Volatile Compounds

Headspace volatiles were analyzed by solid phase microextraction (SPME) and gas chromatography/mass spectroscopy (GC/MS). Compounds were identified by the combination of retention times of standards and mass spectroscopy. Identification of compounds by mass spectroscopy MS was performed throughout storage.

Headspace volatile compounds were isolated by SPME with a 65 mm PDMS/DVB fiber. The fiber was inserted into the vial headspace for 30 minutes at 50 °C. SPME has been used for the extraction of volatile compounds from soybean and corn oil (Steenson et al 2002) and linseed and sunflower oil (Jalen et al 2000). Steenson and others (2002) reported that PDMS solid phase performed better than other solid phase materials for the extraction of volatile chemicals from soybean and corn oil. Jelen and others (2000) reported that solid phases containing DVB, carboxen and PDMS were more sensitive to the extraction of volatile compounds from linseed and sunflower oil. Callison (2000) and Lee (2002) reported that PDMS/DVB was more sensitive than PDMS for the extraction of volatile compounds from soybean oil and linoleic acid.

Extracted volatiles were desorbed in the GC injection port for 5 minutes at 250 °C. The injection port was fitted with a 0.75 mm internal diameter splitless glass liner (Scheppers Wercinski 1999). Volatile compounds were separated and detected using an SPB-5 column (5% diphenyl / 95% dimethylsiloxane) 30 m x 0.25 mm x 0.25 mm film thickness (Supelco) in a flame ionization detector equipped Hewlett-Packard 6890 GC (Wilmington, DE) using helium carrier gas. The GC was programmed to hold at 60 °C for 5 minutes, increase to 120 at 4 °C/minute, increase to 240 at 10 °C/minute and hold at 240 °C for 2 minutes.

Analysis of Data

Four trials were run at each of the three storage temperatures. Headspace volatiles were analyzed after 0, 1, 2, 4 and 7 days of storage. One way ANOVA, using Minitab, was used to determine if storage time, storage temperature and chlorophyll level

were significant factors in the production of total volatile compounds. Means were compared using Tukey's studentized range ($\alpha = 0.05$). T-tests (two-tail, two-sample equal variance) comparing the amounts of each compound at a given set of conditions was calculated in Excel.

The effect of light on the production of volatile compounds was characterized by comparison of the soybean oil containing 0 ppm chlorophyll that was stored in the dark to the soybean oil containing 0 ppm chlorophyll that was exposed to light. The effect of chlorophyll on the production of volatile compounds was characterized by comparison of the soybean oil containing 0 ppm added chlorophyll to the soybean oil that contains 1 ppm or 10 ppm added chlorophyll, all of which were exposed to light. The effect of temperature was determined by the comparison of the volatiles in soybean oil stored at 4, 20 and 40 ^{o}C.

Results and Discussion

Total Volatiles

The soybean oil initially contained relatively few volatile compounds (Figure 3.2). The soybean oil stored in the dark showed no significant increase ($p>0.05$) in the amount of volatile compounds when stored at 4 °C and 20 °C, however, total volatiles increased by 30% in the soybean oil stored in the dark at 40 °C for 7 days. Coefficient of variation for the amounts of volatile compounds in soybean oil was typically 5% to 15%. Coefficient of variation for the amount of total volatiles was approximately 10%. Preliminary trials confirmed that chlorophyll concentration has no affect on the amount

of volatiles in soybean oil stored in the dark throughout the duration of storage at 4, 20 or 40 °C (data not shown).

When the soybean oil containing 0 ppm to 10 ppm chlorophyll was exposed to light at 4, 20 or 40 °C, there was a significant increase in the amount of total volatiles present compared to the amounts present in the soybean oil stored in the dark (Figure 3.3). The total volatile peak area increased over time in a linear manner in the oil containing 0 ppm chlorophyll when stored under light at 4 °C, 20 °C or 40 °C (R^2 > 0.91). The rate increase in total volatile peak area in soybean oil containing 0 ppm chlorophyll was 584/hr at 4 °C and 3248/hr at 40 °C (Table 3.1). The rate of volatile production at 40 °C was 5.5 times greater that that at 4 °C, indicating the significance of storage temperature to the oxidation and production of volatiles in soybean oil. The effect of increased storage temperature to the production of volatiles is evident in the chromatograms (Figure 3.4). It is apparent that light exposure alone was a significant contributor to the oxidation and volatile production of soybean oil. A tungsten light bulb gives off light with a continuous spectrum from 300 nm to 1400 nm. The energy associated with a wavelength of 300 nm is 95 kcal/mol. This energy contributes to the increased rate of oxidation of the soybean oil when exposed to light.

The addition of 5 ppm or 10 ppm chlorophyll significantly affected the amount of total volatiles in the soybean oil stored at 4 °C, 20 °C or 40 °C (Figure 3.3). The amount of total volatiles in the oil containing 1 ppm chlorophyll was not statistically different from the amount in the oil containing 0 ppm chlorophyll stored at 4 °C or 40 °C. The peak area increase in the soybean oil containing 1 ppm, 5 ppm or 10 ppm chlorophyll stored at 4 °C was 598/hr, 858/hr and 1065/hr, respectively (Table 3.1). Therefore,

production rate of volatile compounds in the soybean oil containing 10 ppm chlorophyll was approximately 2 times as fast as the production of total volatiles in the oil containing 0 ppm chlorophyll that was exposed to light.

Effect of Light Exposure

Compounds that are formed by autoxidation mechanisms would be expected to be significantly greater in quantity in the soybean oil that is exposed to light due to the energy provided by light. T-tests between the amounts each compound in the soybean oil containing 0 ppm added chlorophyll and stored in the dark or exposed to light was calculated after 1, 2, 4 and 7 days of storage to determine if the compound was significantly affected by light exposure. All of the compounds measured in soybean oil were significantly affected ($p<0.05$) by light exposure at 4 °C, 20 °C or 40 °C (Table 3.2). These results indicate that all of the volatile compounds identified in the soybean oil were formed in significant amounts by triplet oxygen autoxidation mechanisms.

Effect of Chlorophyll

As shown in table 3.2, most of the volatile compounds identified in soybean oil were significantly affected by the presence of 10 ppm chlorophyll after 7 days of storage. Similar results were obtained from the oil containing 5 ppm chlorophyll. Since singlet oxygen oxidation can produce free-radicals and accelerate autoxidation, these results do not indicate that these compounds are primary products of singlet oxygen oxidation. To further explore this, t-tests were calculated between the means of the amount of each compound in the oil containing 0 ppm added chlorophyll and the amount in the oil

containing 1 ppm, 5 ppm or 10 ppm added chlorophyll after 24 hours storage at 4, 20 and 40 °C. Only pentanal and 1-pentenol were significantly affected by 1 ppm chlorophyll at two of the three storage temperatures (Table 3.3). Approximately half of the compounds identified were significantly affected by 5 and 10 ppm chlorophyll after 1 day of storage. These compounds are likely products of singlet oxygen oxidation. Soybean oil with or without added chlorophyll were qualitatively similar in volatile compound composition.

After 1 day of storage, the peak area increase in total volatiles in the soybean oil exposed to light containing 10 ppm chlorophyll was 3, 4 and 7 times the amount of total volatiles in the soybean oil exposed to light containing 0 ppm chlorophyll stored at 4, 20 and 40 °C, respectively (Table 3.2). Several low molecular weight compounds were the major contributor to the increase in total volatiles after 1 day of light exposure (Figure 3.5). After 7 days of storage, the amount of total volatiles in the soybean oil containing 10 ppm chlorophyll was approximately 2 times the amount of total volatiles in the oil containing 0 ppm chlorophyll stored at 4, 20 and 40 °C, respectively. The greater disparity in amounts of total volatiles in the 0 ppm and 10 ppm chlorophyll oil after 1 day of storage is indicative of the activity of singlet oxygen oxidation. Since the rate of singlet oxygen oxidation is much greater than the rate of autoxidation, the effect of chlorophyll concentration on volatile production would be expected to be most prominent early in storage, before autoxidation mechanisms can greatly contribute to the production of volatile compounds.

Hexanal

The amount of hexanal in soybean oil is commonly used as an indicator of oxidation and quality loss since it is a primary product of autoxidation of linoleic acid, the most abundant fatty acid in soybean oil (Figure 3.6).

Hexanal was initially present in the soybean oil and was one of the most abundant compounds in the oil throughout the storage. Light exposure and storage temperature were significant factors in the production of hexanal. The amount of hexanal increased over time in a linear manner in the oil containing 0 ppm chlorophyll when stored under light at 4 °C, 20 °C or 40 °C during the first 4 days of storage (Figure 3.7). The increase in the hexanal peak area in the linear phase was 65/hr to 131/hr when stored at 4 °C or 20 °C (Table 3.1). Storage at 40 °C resulted in production rates of 385/hr to 429/hr. The addition of 1 to 10 ppm chlorophyll to the soybean oil did not result in increases in the amount of hexanal in the oil when compared to the oil containing no added chlorophyll. This indicates that hexanal is not formed in significant amounts by singlet oxygen oxidation in soybean oil relative to formation by triplet oxygen oxidation.

2-Heptenal

2-Heptenal can be formed by direct addition of singlet oxygen with linoleic acid (Figure 3.8). 2-Heptenal was not present in the soybean oil initially and was greatest in the in the soybean oil that contained 10 ppm added chlorophyll and had been stored for 7 days at 40 °C. The amount of 2-heptenal increased over time in a linear manner (R^2 > 0.85) in the oil containing 0 ppm chlorophyll when stored under light at 4 °C, 20 °C or 40

°C (Figure 3.9). The increase in 2-heptenal in the 0 ppm oil exposed to light was 37/hr at 4 °C and 520/hr at 40 °C (Table 3.1).

The addition of chlorophyll to the soybean oil significantly affected the amount of 2-heptenal in the oil stored at 4 °C, 20 °C or 40 °C. The increase of 2-heptenal in the soybean oil containing 1 ppm, 5 ppm or 10 ppm chlorophyll stored at 4 °C was 58/hr, 140/hr and 219/hr, respectively (Table 3.1). This formation rate is several times greater than the formation rate of the triplet oxygen product hexanal at 4 °C. Increase of 2-heptenal in the soybean oil containing 1 ppm, 5 ppm or 10 ppm chlorophyll stored at 40 °C was 908/hr, 1971/hr and 3030/hr, respectively. Clearly, the concentrations of chlorophyll and storage temperature are significant factors in the production of 2-heptenal in the soybean oil. Through the first 2 days of storage the amount of 2-heptenal in the 10 ppm soybean oil was over 10 times the amount present in the soybean oil containing 0 ppm chlorophyll. The disparity was most prominent in the oil stored at 40 °C, where the amount of 2-heptenal in the 10 ppm chlorophyll oil was 50 times greater than the amount in the 0 ppm chlorophyll after 24 of light exposure. After 7 days of storage at 40 °C, the amount of 2-heptenal in the 10 ppm soybean oil was less than 5 times the amount in the 0 ppm oil. The diminishing disparity in the amount of 2-heptenal formed in soybean oil containing added chlorophyll is similar to that observed in the production of total volatiles. Chlorophyll concentration is a more significant factor in the production of 2-heptenal and total volatiles during early storage.

3.5. Conclusions

The amount of total volatile compounds produced in soybean oil was dependant on storage time, storage temperature, light exposure and chlorophyll concentration. The effect of chlorophyll concentration on the amount of total volatiles present was most prominent during the first 2 days of storage. Light exposure had a significant effect on the production of all volatile compounds in soybean oil stored for 7 days at 4, 20 or 40 °C. Volatiles present in soybean oil with or without added chlorophyll were qualitatively similar.

Chlorophyll concentrations of 5 and 10 ppm present in soybean oil significantly increased the production of approximately half of the volatile compounds in the soybean oil after 2 days of storage. These compounds are likely primary or secondary products of singlet oxygen oxidation. Chlorophyll concentration had no effect on the amount of the triplet oxygen autoxidation product hexanal. The amount of hexanal in soybean oil was dependant on storage temperature, light exposure and the duration of storage. The storage temperature, chlorophyll concentration and light exposure were significant factors in the production of total volatiles and the singlet oxygen product 2-heptenal. 2-Heptenal, which was not present in the oil initially increased in quantity rapidly at 4 °C when the soybean oil containing 5 ppm or 10 ppm chlorophyll was exposed to light. The rate of increase of 2-heptenal was several times greater than the rate of increase of hexanal in soybean oil containing 5 ppm or 10 ppm chlorophyll. The effect of chlorophyll on the amount of 2-heptenal was most prominent during the first 2 days of storage. Amounts of 2-heptenal in oil containing 10 ppm chlorophyll was approximately

10 times greater than the oil containing 0 ppm chlorophyll during the first 2 days of storage.

Storage at 40 °C resulted in the greatest increase in volatile production. However, this was dependant on the soybean oil being exposed to light. The presence of chlorophyll was the most significant factor to the amount of chlorophyll sensitive compounds produced during the first 2 days of storage; however, temperature was the most significant factor after 2 days of storage.

Exposure of foods containing lipids and photosensitizers to light can have detrimental effects on the production of off-flavors in those foods, even if the food is exposed to light for short periods of time at refrigerated temperatures.

	Total Volatiles			[1]Hexanal			2-Heptenal		
	4 °C	20 °C	40 °C	4 °C	20 °C	40 °C	4 °C	20 °C	40 °C
0 ppm	583	496	3248	131	79	429	37	32	520
1 ppm	599	749	3806	99	80	385	58	62	908
5 ppm	858	1094	5636	65	75	426	140	169	1971
10 ppm	1066	1321	7363	84	69	409	219	274	3030

[1] Regression based on 4 days of storage. Chlorophyll was not a significant factor in the production of hexanal.

Table 3.1. Increase in total volatiles, hexanal and 2-heptenal (peak area / hour) in soybean oil stored at 4 °C, 20 °C and 40 °C for 7 days. R^2 for regression were >0.86.

Compound Identification	Initial	Storage at 4 °C			Storage at 20 °C			Storage at 40 °C		
		0 ppm Dark	0 ppm Light	10 ppm Light	Dark Dark	0 ppm Light	10 ppm Light	Dark Dark	0 ppm Light	10 ppm Light
Pentane	787	978	**2121**	**8777**	744	**2375**	**10120**	1465	**17581**	**27774**
1-Hexene	228	ND	**3298**	**4483**	425	**3054**	**5376**	701	**15982**	**40227**
Hexane	ND	ND	**1009**	**1770**	ND	**1198**	**2595**	ND	**24720**	**44450**
Pentanal	776	940	**27549**	**63417**	837	**29013**	**76512**	1627	**71903**	74715
2-Pentenal	ND	ND	**551**	712	ND	**507**	**994**	ND	**6817**	**7499**
1-Pentanol	880	894	**3718**	**5151**	417	**3396**	**5726**	ND	**14266**	**18299**
1-Octene	703	441	**1504**	1629	1488	1535	**2064**	3455	607	**1651**
Hexanal	16157	13734	**27624**	25387	17145	**28817**	**28825**	19597	**79834**	**67555**
1-Nonene	1019	882	**1257**	1074	987	**1290**	1228	ND	1371	**2625**
2-Hexenal	477	ND	**2261**	**2758**	ND	**1939**	**3630**	ND	ND	ND
Heptanal	1933	1856	**2553**	2136	1135	**2297**	2060	1047	**6514**	**2706**
2-heptenal	ND	408	**6363**	**36514**	ND	**5569**	**45550**	ND	**104883**	**516801**
1-Octen-3-ol	1904	2569	**ND**	**3811**	ND	**4441**	5039	686	**28783**	**145707**
2-Pentyl Furan	725	813	**1290**	1041	720	**1089**	1206	720	**3129**	**2399**
2,4-heptadienal	ND	ND	ND	**719**	13240	13847	**5445**	ND	**18220**	**24658**
Undecane	ND	394	**2704**	2613	ND	**2025**	**1403**	ND	**4446**	**3823**
Nonanal	453	628	**2087**	1978	398	**1725**	**1413**	ND	**3309**	**3582**
2,4-nonadienal	3663	3585	**4075**	**3529**	3296	3672	3366	3490	**4216**	**3579**
Total Volatiles	65251	65904	**150200**	**234743**	68796	**148378**	**274345**	81173	**654935**	**1300418**

ND = indicates that the compounds was not detected at these conditions
Peak areas of compounds that are significantly different (0 ppm dark vs 0 ppm light and 0 ppm light vs 10 ppm light) are shown in bold

Table 3.2. Average peak areas of volatile compounds in soybean oil containing 0 ppm or 10 ppm chlorophyll after 7 days of storage in the dark or under light at 4, 20 ro 40 °C.

Compound Identification	Significantly Affected by Indicated Chlorophyll Concentration		
	1 ppm	5 ppm	10 ppm
Pentane		Yes	Yes
1-Hexene			Yes
Hexane			Yes
Pentanal	Yes	Yes	Yes
2-Pentenal			Yes
1-Pentanol	Yes	Yes	Yes
Heptanal			Yes
2-heptenal		Yes	Yes
1-Octen-3-ol		Yes	Yes
2-Pentyl Furan		Yes	Yes
2,4-heptadienal			Yes
2,4-nonadienal		Yes	
Total Volatiles	Yes	Yes	Yes

Table 3.3. Conpounds that were significantly affected by 1, 5 or 10 ppm added chlorophyll to soybean oil stored at 4, 20 or 40 ^{o}C for 1 day.

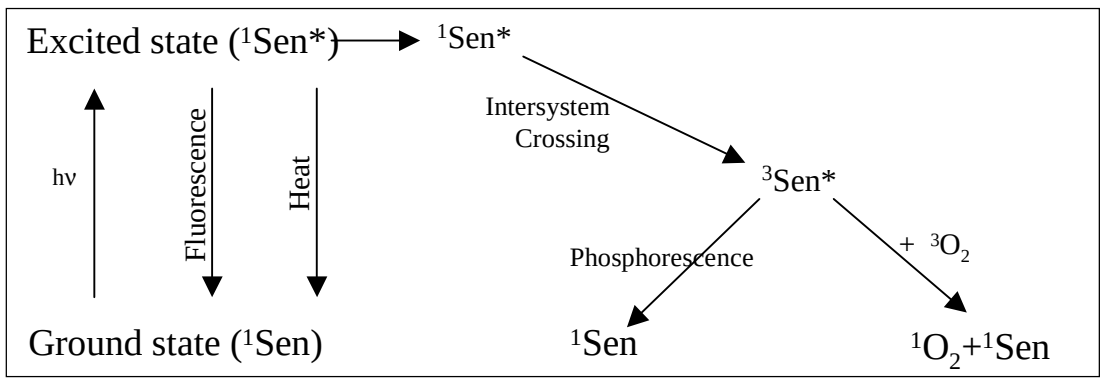


Figure 3.1. Description of the formation of singlet oxygen in the presence of sensitizer, light and triplet oxygen showing the excitation and deactivation of photosensitizer in the formation of singlet oxygen (Sharman and others 2000).

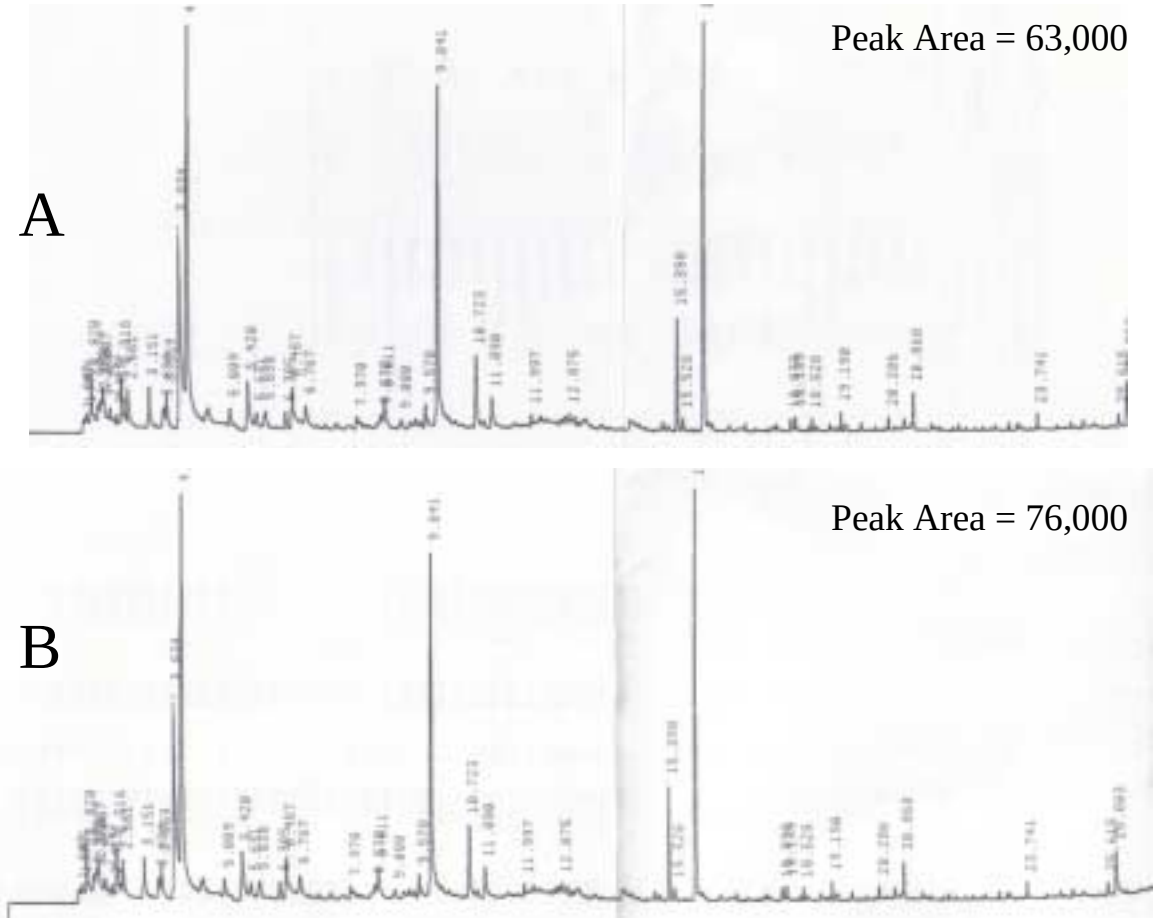


Figure 3.2. Volatile compounds in soybean oil stored in the dark at 40 ºC after (A) 0 days, (B) 7 days.

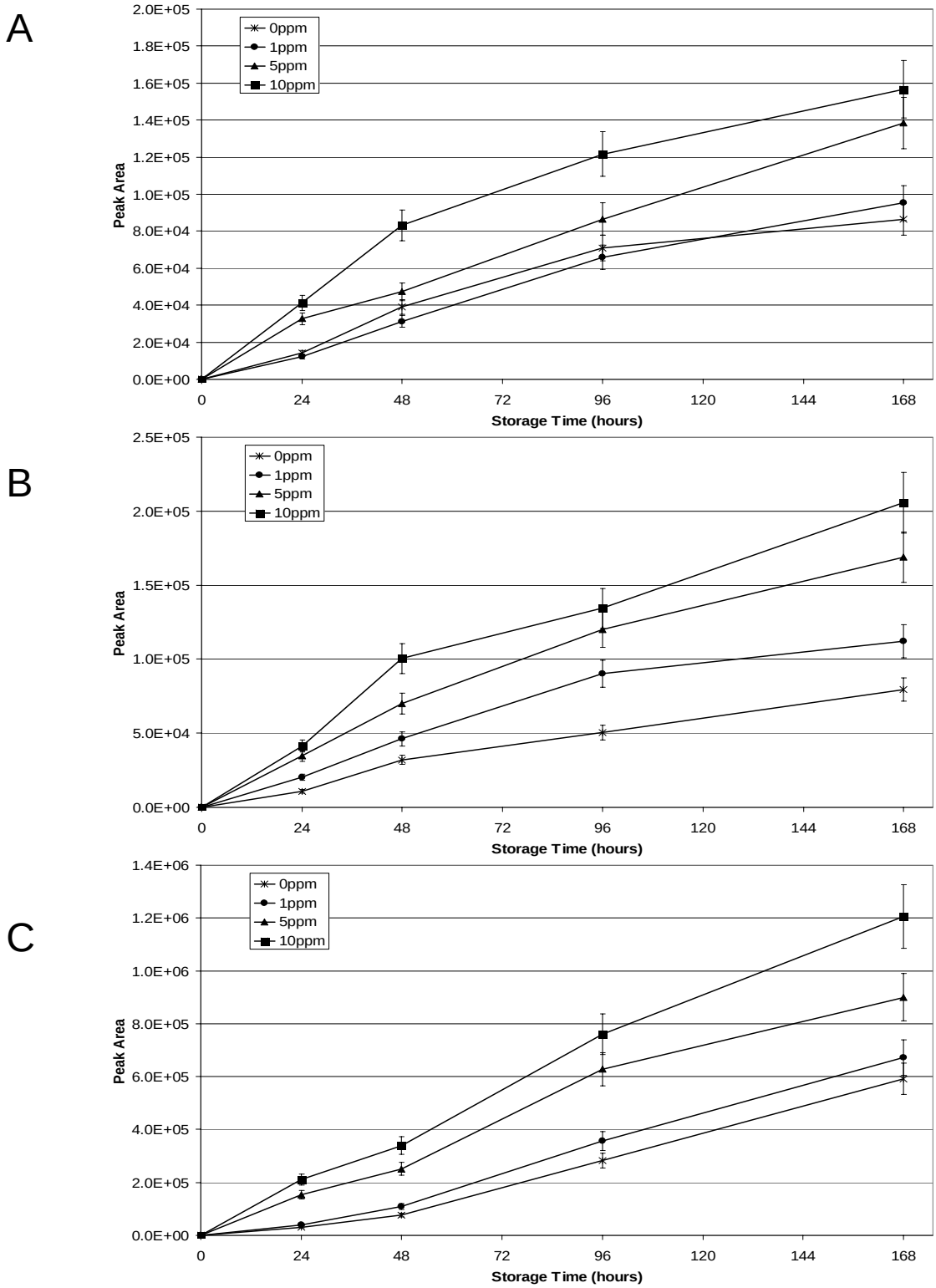


Figure 3.3. Total volatiles in soybean oil containing 0ppm, 1ppm, 5ppm or 10ppm chlorophyll and exposed to light for 7 days at (A) 4 ºC, (B) 20 ºC and (C) 40 ºC. The amount of volatiles does not change when stored in the dark.

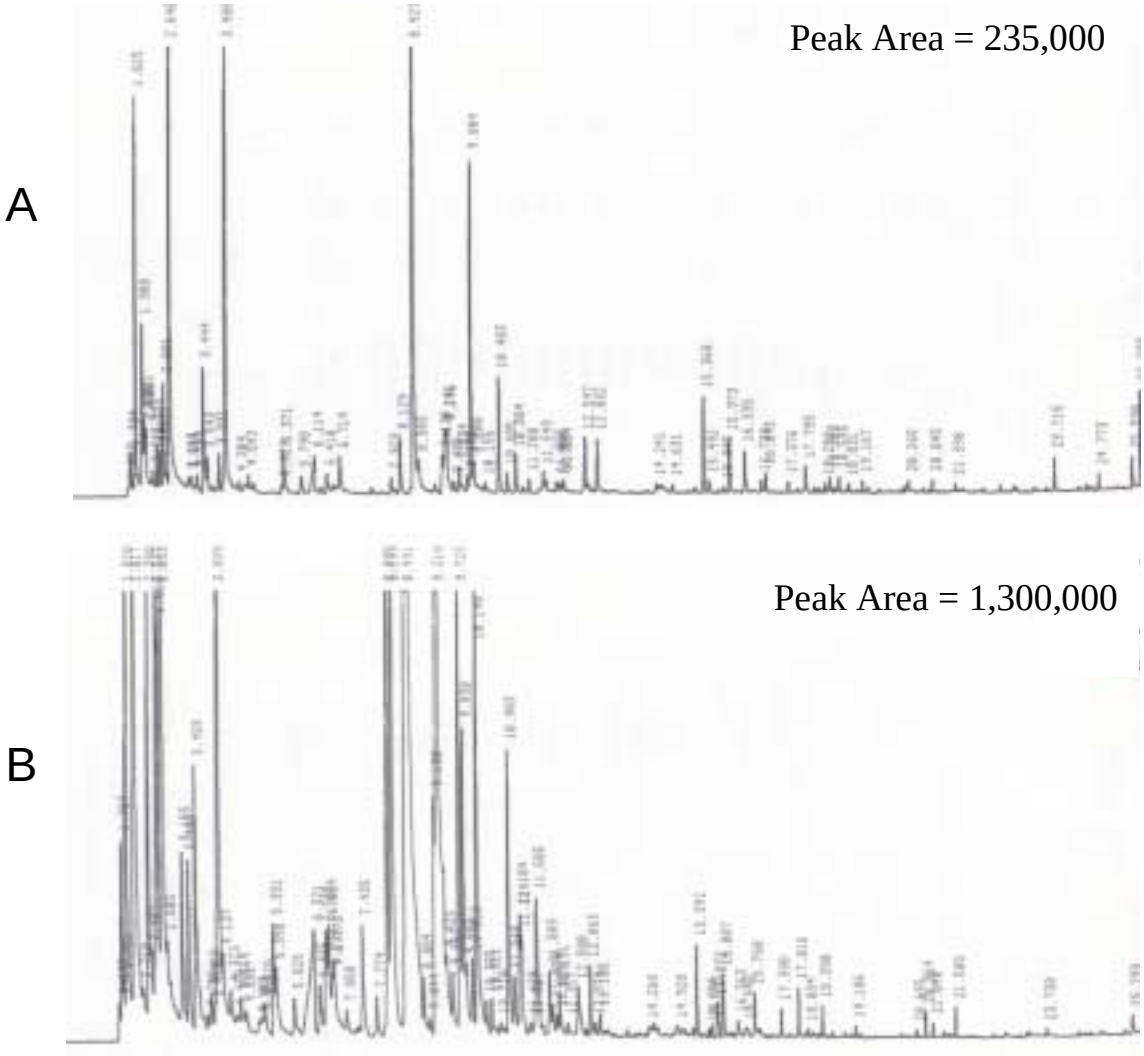


Figure 3.4. Gas chromatograms of headspace volatiles in soybean oil containing 10 ppm chlorophyll after 7 days light exposure at (A) 4 ^{o}C and (B) 40 ^{o}C.

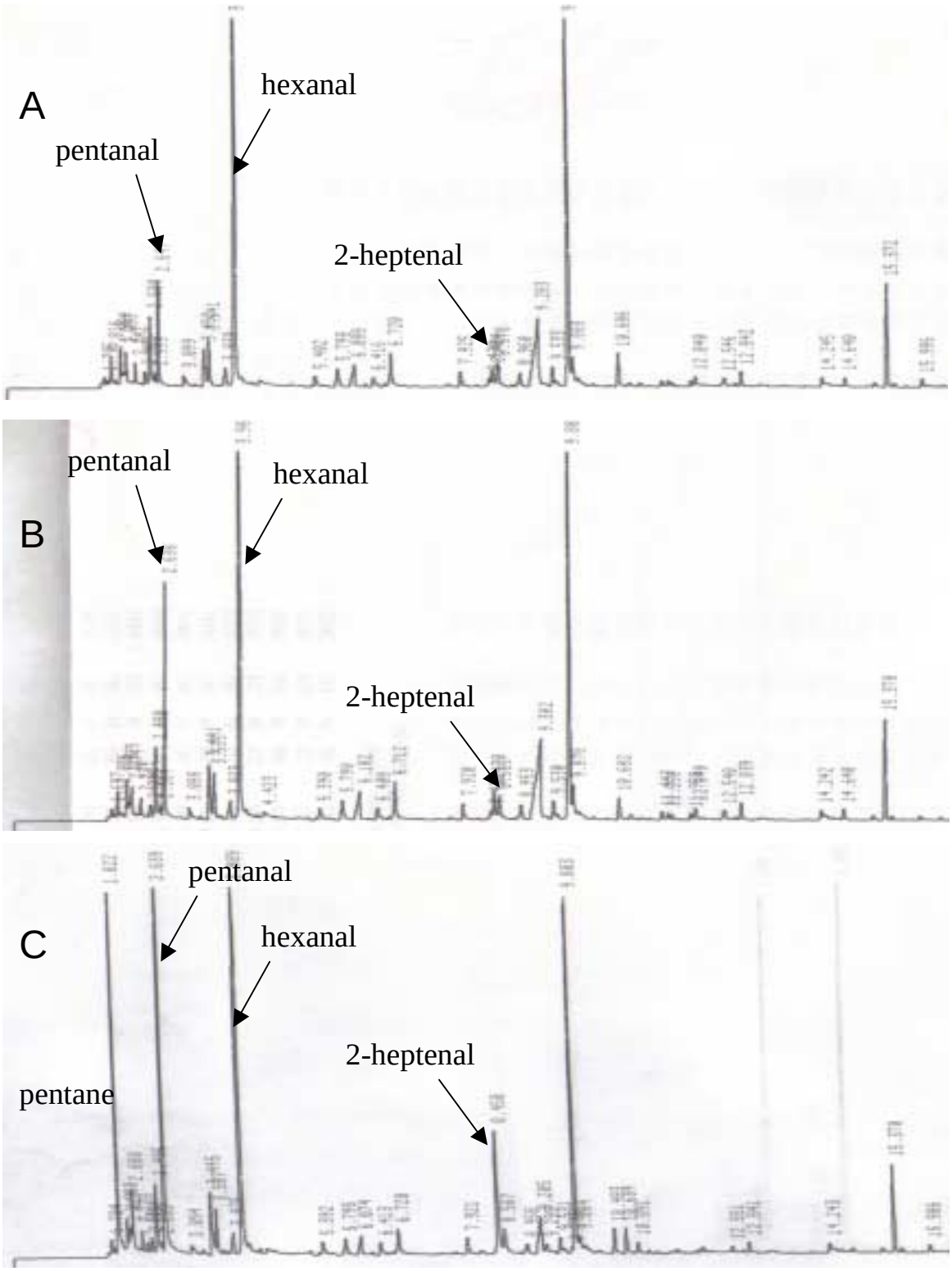


Figure 3.5. Volatile compounds in soybean oil exposed to light at 4 ºC after 1 day, containing (A) 0 ppm, (B) 1 ppm, or (C) 10 ppm chlorophyll.

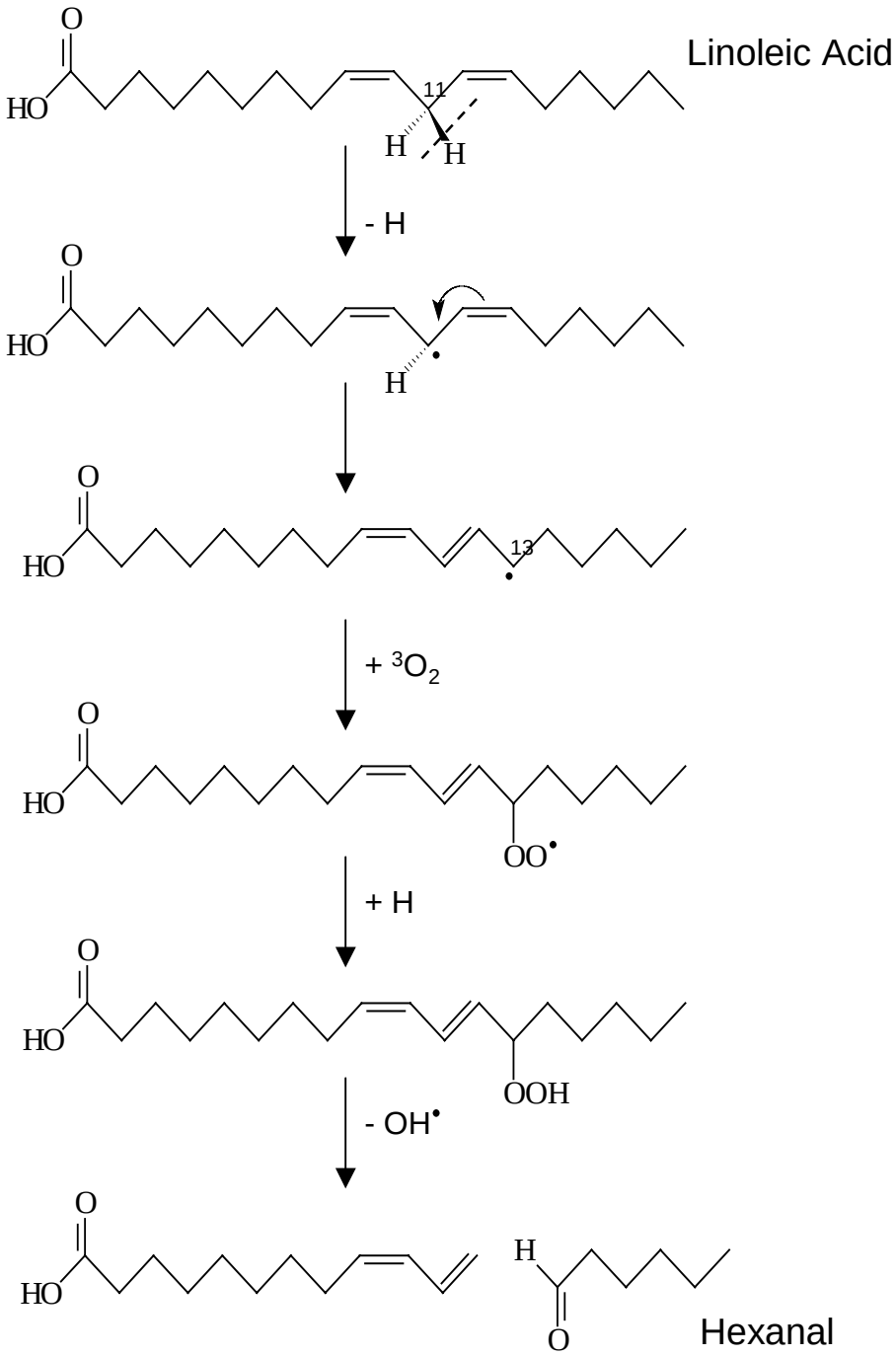


Figure 3.6. Formation of hexanal from linoleic acid by triplet oxygen autoxidation.

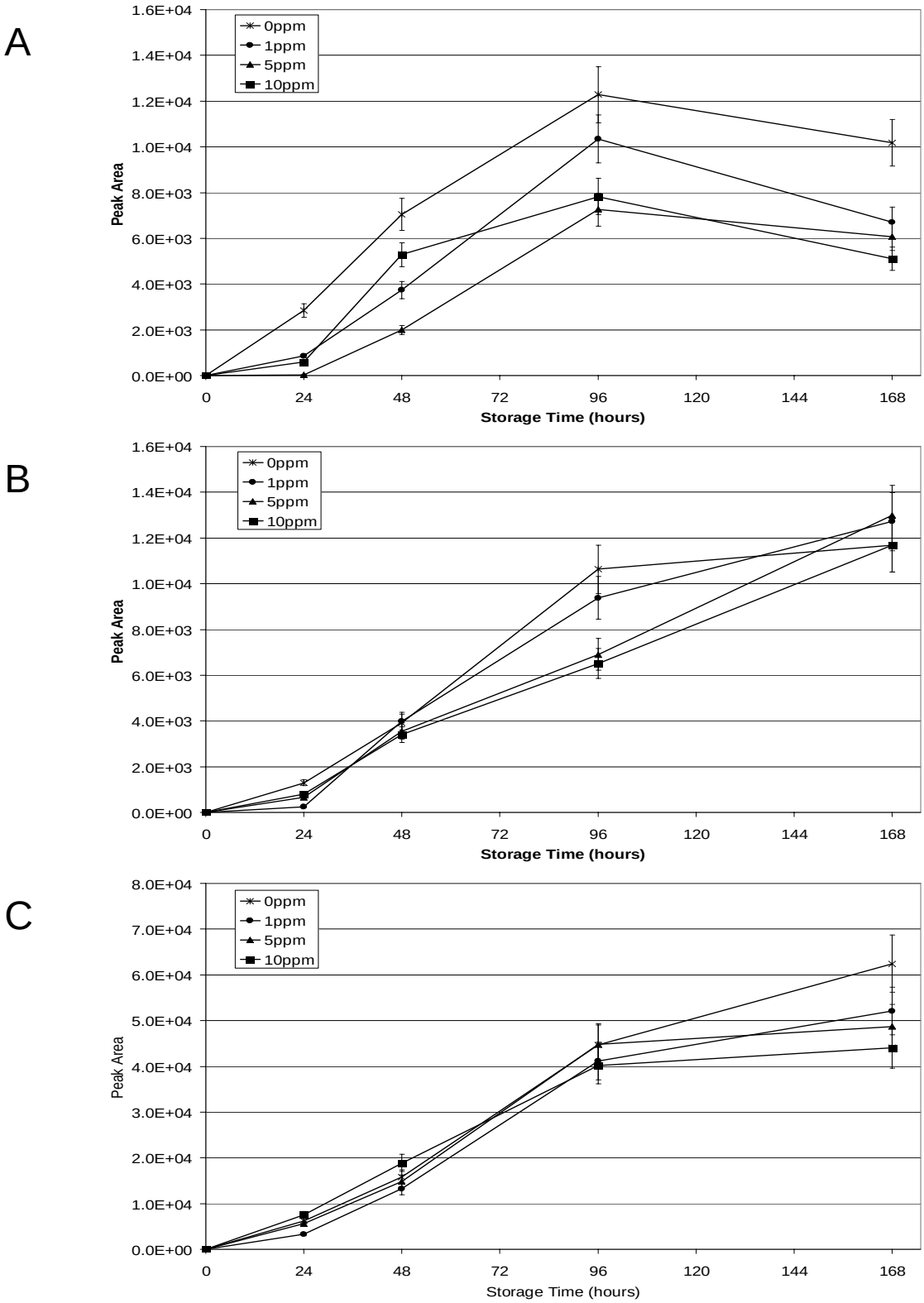


Figure 3.7. Hexanal in soybean oil containing 0ppm, 1ppm, 5ppm or 10ppm chlorophyll and exposed to light for 7 days at (A) 4C, (B) 20C and (C) 40C.

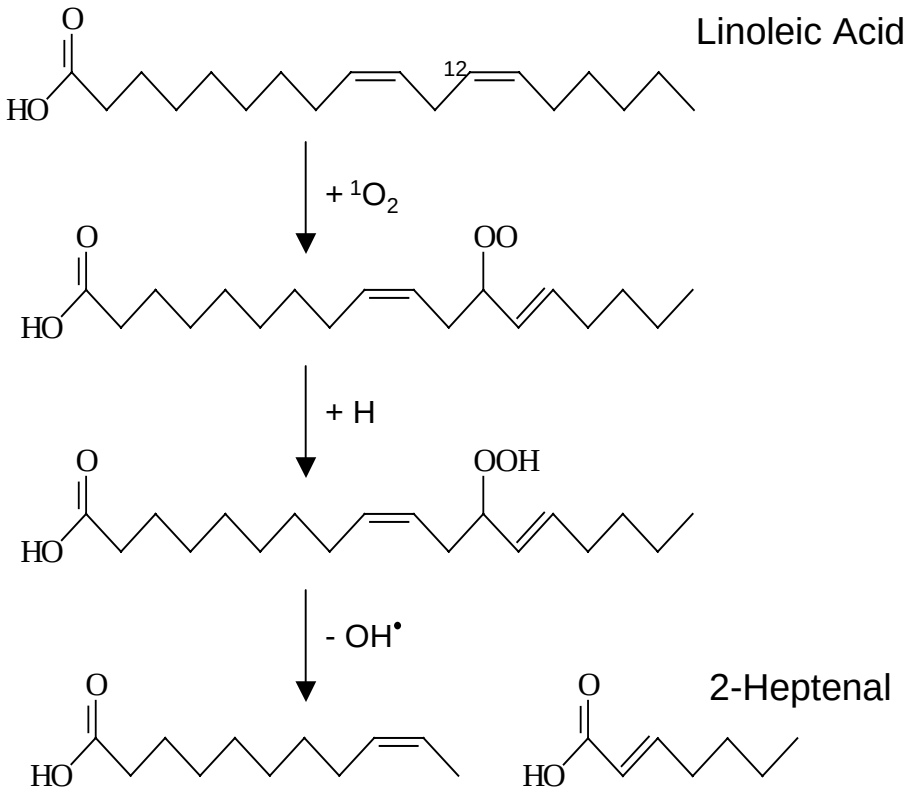


Figure 3.8. Formation of 2-heptenal from linoleic acid by singlet oxygen autoxidation.

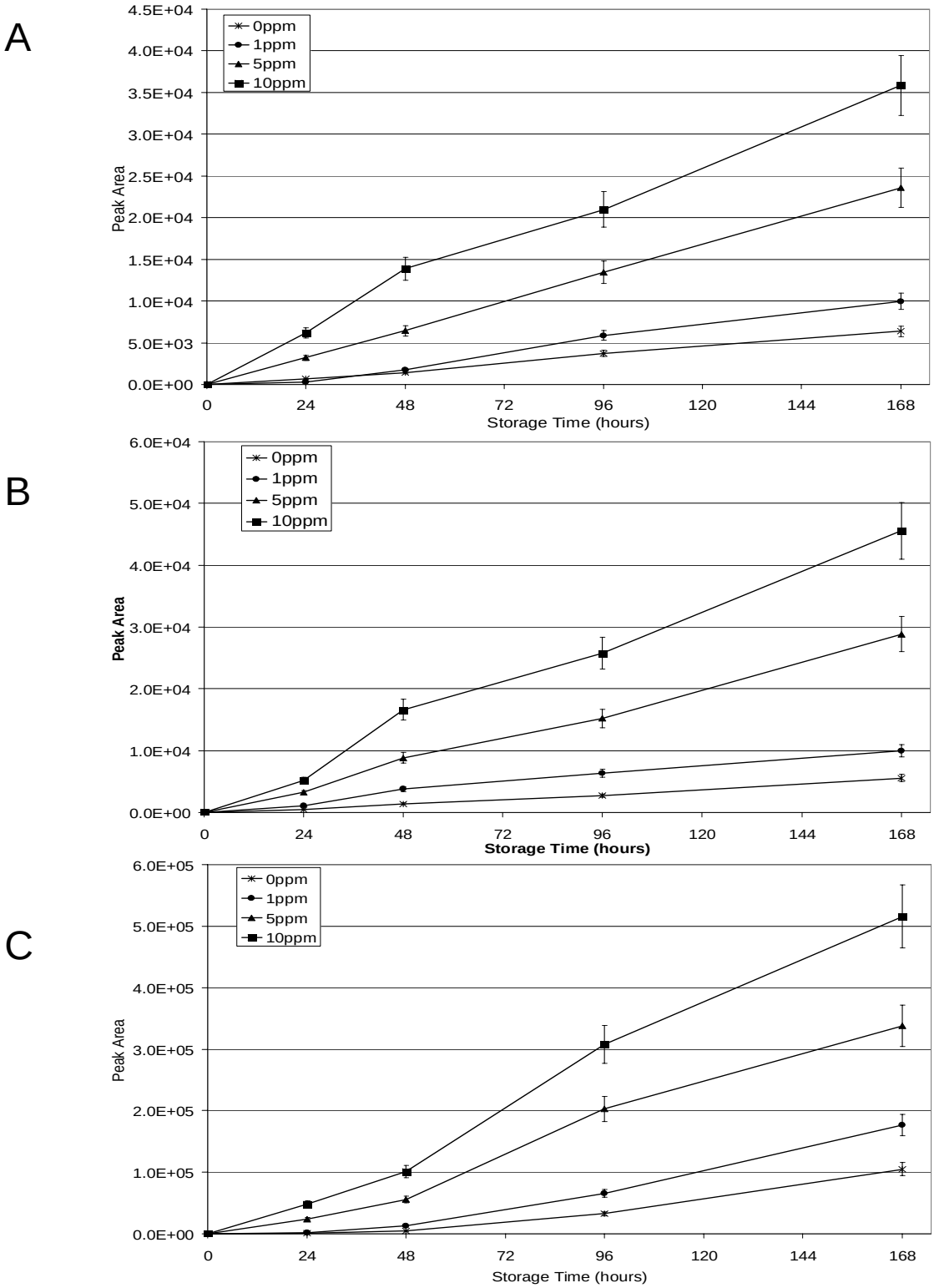


Figure 3.9. 2-Heptenal in soybean oil containing 0ppm, 1ppm, 5ppm or 10ppm chlorophyll and exposed to light for 7 days at (A) 4C, (B) 20C and (C) 40C. 2-Heptenal did not increase in oil stored in the dark.

CHAPTER 4

PHOTOXIDATION AND PHOTOSENSITIZED OXIDATION OF PEANUT OIL

4.1 Abstract

Chlorophyll photosensitized oxidation can increase the rate of oxidation and development of volatile compounds in edible oils, thereby reducing product quality. It is desirable to study the effect of photosensitized oxidation in a sensitizer-free lipid such as peanut oil. The primary objective of this research was to determine the effect of fatty acid composition on the production of volatiles by comparison to the results obtained from the soybean oil study. Peanut oil containing 0, 1, or 10 ppm chlorophyll was stored under light at 4 and 40 °C for 10 days. Volatile compounds were analyzed by solid phase microextraction and gas chromatography.

Light exposure had a significant effect on the production of all volatile compounds in peanut oil stored in the light for 10 days at 4 or 40 °C. The presence of 10 chlorophyll in peanut oil significantly increased the production of over half of the volatile compounds in peanut oil stored at 40 °C for 10 days. The increase in the formation of total volatiles, hexanal and 2-heptenal in the soybean oil stored at 40 °C was

approximately 4 to 4.5 times greater than the increases in peanut oil stored at 40 °C. This is likely due in part to the differences in fatty acid composition of peanut oil and soybean oil. These results indicate that fatty acid composition is also an important factor in photoxidation as well and that autoxidation is an important component of volatile production resulting from photosensitized reactions.

4.2 Introduction

Many oils derived from plants can contain chlorophyll (Swern 1979; Brekke 1980; Koseoglu and Lusas 1990). This has been recognized by manufacturers, and refining methods to remove pigmentation adequately eliminates nearly all of the chlorophyll from edible oils. However, it is difficult to remove all of the chlorophyll, refined peanut oil may still contain 40 to 50 ppb chlorophyll. It is desirable to study the effect of photosensitized oxidation in a sensitizer-free lipid. Peanut oil does not contain chlorophyll (Swern 1979; Brekke 1980) and was therefore chosen for the present study. Peanut oil contains approximately 21% saturated fatty acids and 79% unsaturated fatty acids (Table 4.1). Approximately 45% of peanut oil is composed of the monounsaturated fatty acid oleic acid. Polyunsaturated linolenic acid accounts for 30 to 35% of the fatty acids in peanut oil. Soybean oil contains 5-7% linolenic acid, which is 2 times as reactive to autoxidation as linoleic acid. Peanut oil contains no linolenic acid. Based on the fatty acid composition, one would expect soybean oil to be more susceptible to autoxidation than peanut oil. Since singlet oxygen reacts with unsaturated fatty acids at a nearly equal rates and the unsaturated fatty acid content in peanut and soybean oils are nearly equal,

singlet oxygen products would not be expected to be as greatly affected by the fatty acid composition as autoxidation products.

The primary objective of this research was to (1) determine the effect of fatty acid composition to the production of volatiles by comparison to the results obtained from the soybean oil study and (2) determine the effects of chlorophyll concentration on the production of volatile compounds in peanut oil.

4.3 Materials and Methods

Materials

Planters peanut oil was purchased from Kroger supermarket (Columbus, Ohio). Chlorophyll b and the chemical standards 1–pentenol, hexanal, 2-hexenal, heptanal, 2-heptenal, 1-octen-3-ol and 2,4-heptadienal were purchased from Sigma Chemical Co. (St.Louis, MO). The chemical standard 2-pentyl furan was purchased from Karl Industries Inc. (Aurora, OH). The gas chromatography (GC) columns, the polydimethylsiloxane/divinylbenzene (PDMS/DVB) solid phase microextraction fibers, fiber holder, glass GC inlet liners, 10ml clear glass serum vials, Teflon-coated rubber septa and aluminum crimp caps were purchased from Supelco, Inc. (Bellefonte, PA). The chlorophyll b was stored at –20 ^{o}C until use. Other chemicals were stored at 4 ^{o}C.

Sample Preparation and Storage

Chlorophyll was added to the peanut oil at concentrations of 1 and 10 ppm and mixed with a stirring bar for 4 hours at room temperature in a sealed flask that had been flushed with nitrogen. A 2 ml aliquot of the prepared peanut oil containing 0, 1, or 10

ppm added chlorophyll was placed in a 10 ml glass vial and sealed with a Teflon-coated rubber septa and aluminum crimp cap in order to prevent contamination of the vial headspace. The prepared vials of peanut oil were stored in a mirrored light box for 240 hours while exposed to a tungsten light (1560 lumens). The temperature in the light box was maintained at 4 (±1 ^{o}C) or 40 ^{o}C (±2 ^{o}C) throughout the duration of the storage. A 2 ml aliquot of peanut oil containing 0 ppm chlorophyll was wrapped with foil to prevent light exposure and stored in the light box for 240 hours along with the other vials. Prior research has established that chlorophyll has no effect on oil quality if the oil is not exposed to light (Lee 2003); therefore, the oil stored in the dark did not contain added chlorophyll.

Analysis of Volatile Compounds

Headspace volatiles were analyzed by solid phase microextraction (SPME) and gas chromatography/mass spectroscopy (GC/MS). Compounds were identified by the combination of retention times of standards and mass spectroscopy. Identification of compounds by mass spectroscopy MS was performed throughout storage.

Volatile compounds were extracted via headspace-SPME using a 65mm PDMS/DVB coated fiber by manual insertion of the fiber into the vial headspace for 30 minutes at 50 ^{o}C. SPME has been used for the extraction of volatile compounds from peanut and corn oil (Steenson et al 2002) and linseed and sunflower oil (Jalen et al 2000). Steenson and others (2002) reported that PDMS solid phase performed better than other solid phase materials for the extraction of volatile chemicals from peanut and corn oil. Jelen and others (2000) reported that solid phases containing DVB, carboxen and PDMS

were more sensitive to the extraction of volatile compounds from linseed and sunflower oil. Callison (2000) and Lee (2002) reported that PDMS/DVB was more sensitive than PDMS for the extraction of volatile compounds from peanut oil and linoleic acid, respectively.

Extracted volatiles were desorbed in the GC injection port for 5 minutes at 250 ^{o}C. The injection port was fitted with a 0.75 mm internal diameter splitless glass liner (Scheppers Wercinski 1999). Volatile compounds were separated and detected using an SPB-5 column (5% diphenyl / 95% dimethylsiloxane) 30 m x 0.25 mm x 0.25 mm film thickness (Supelco) in a flame ionization detector equipped Hewlett-Packard 6890 GC (Wilmington, DE) using helium carrier gas. The GC was programmed to hold at 60 ^{o}C for 5 minutes, increase to 120 at 4 ^{o}C /minute, increase to 240 at 10 ^{o}C /minute and hold at 240 ^{o}C for 2 minutes.

Analysis of Data

Three trials were run at each temperature. For each trail stored at 40 ^{o}C, headspace volatiles were collected and analyzed after 0, 1, 2, 4, 7 and 10 days of storage. For each sample set stored after 4 ^{o}C, headspace volatiles were collected and analyzed after 0, 1 and 10 days of storage. One way ANOVA, using Minitab, was used to determine if storage time, storage temperature, light exposure and chlorophyll level were significant factors in the production of volatile compounds. Means of significant factors were compared using Tukey's studentized range ($\alpha = 0.05$). T-tests (two-tail, two-sample equal variance) were used for direct comparison of the amounts of each compound produced and calculated using Excel.

The effect of light on the production of volatile compounds was characterized by comparison of the peanut oil containing 0 ppm chlorophyll that was stored in the dark to the peanut oil containing 0 ppm chlorophyll that was exposed to light. The effect of chlorophyll on the production of volatile compounds was characterized by comparison of the peanut oil containing 0 ppm added chlorophyll to the peanut oil that contains 1 ppm or 10 ppm added chlorophyll, all of which are stored under light. The effect of temperature was determined by the comparison of the volatiles in peanut oil stored at 4 ^{o}C and 40 ^{o}C.

4.4 Results and Discussion

Total Volatiles

The peanut oil initially contained few volatile compounds (Figure 4.1). Storage at 40 °C in the dark for 10 days resulted in a significant increase in the amount of total volatiles in peanut oil. Coefficients of variation for the amount of compounds produced in peanut oil were typically 5% to 15%. ANOVA showed that light exposure, storage time, storage temperature and the amount of added chlorophyll were all significant factors ($p<0.05$) in the production of total volatiles in peanut oil.

When the peanut oil containing 0 ppm to 10 ppm chlorophyll was exposed to light, there was a significant increase in the amount of total volatiles compared to the amounts present in the peanut oil stored in the dark (Figure 4.2). The amount of total volatiles in peanut oil exposed to light containing 0 ppm and 1 ppm chlorophyll was statistically similar. The amount of total volatiles increased over time from an initial peak area of 70,000 in a linear manner with a peak area rate increase of 693/hr in the oil

containing 0 ppm chlorophyll when stored under light at 40 °C (R^2 = 0.98). The peak area increase in soybean oil was approximately 4.5 times as large as this (Table 3.1). This is likely due to the higher polyunsaturated fatty acid composition of soybean oil.

The presence of 10 ppm chlorophyll in the peanut oil significantly affected the amount of total volatiles in the oil stored at 4 °C and 40 °C ($p<0.05$). The amount of total volatiles in the peanut oil containing 1 ppm chlorophyll was not statistically different from the amount in the oil containing 0 ppm added chlorophyll stored at 40 °C until the 10^{th} day of storage. However, 1 ppm chlorophyll was a significant factor in amount of total volatiles in the peanut oil stored at 4 °C. Storage at 40 °C results in a greater rate of autoxidation than storage at 4 °C. This is confirmed by the increase in the amount of total volatiles in the peanut oil stored in the dark at 40 °C. The production of volatiles resulting from the addition of 1 ppm chlorophyll is insignificant to the production of volatiles resulting from autoxidation at 40 °C storage, but is significant at 4 °C storage due to the limited rate of autoxidation at 4 °C. The effect of increased storage temperature and chlorophyll concentration to the production of volatiles is evident in the chromatograms (Figure 4.3). The amount of total volatiles increased over time in a linear manner with a peak area increase of 1753/hr and 1013/hr ($R^2 > 0.91$) in the oil containing 10 ppm and 1 ppm chlorophyll, respectively, when stored under light at 40 °C. The peak area increase in soybean oil was approximately 4 times as large (Table 3.1).

Light

T-tests between the amounts each compound in the peanut oil stored in the dark or stored under light containing 0 ppm added chlorophyll were calculated after 1, 2, 4, 7 and

10 days of storage at 40 °C in order to determine if each compound was significantly affected by light exposure. All of the identified compounds were significantly affected by light exposure at 4 or 40 °C storage (Table 4.2). Volatile compounds present in peanut oil with or without added chlorophyll were qualitatively similar. This observation is similar to that observed for the soybean oil. Compounds that are formed by autoxidation mechanisms would be expected to be in significantly greater quantity in the peanut oil that is exposed to light due to the energy provided by light. These results indicate that the volatile compounds identified in peanut oil are formed in significant amounts by autoxidation mechanisms.

Chlorophyll

T-tests between the amounts of each compound in the 0 ppm chlorophyll peanut oil exposed to light and the 1 ppm and 10 ppm chlorophyll peanut oil exposed to light were calculated after 24 hours of storage at 4 °C and 40 °C in order to determine if the compound was significantly affected to the presence of 1 ppm or 10 ppm chlorophyll in the peanut oil.

After 10 days of light exposure at 40 °C, the peanut oil containing 1 ppm chlorophyll had significantly greater quantities of 2-heptenal, 1-octen-3-ol, 2-octenal and undecane compared to the oil containing 0 ppm chlorophyll (Table 4.2). None of the compounds were significantly affected by the addition of 1 ppm chlorophyll to the peanut oil stored at 4 °C. The presence of 10 ppm chlorophyll significantly affected the amounts of over half of the compounds in soybean oil stored at 40 °C for 10 days. Most of the compounds found to be significantly affected by the addition of chlorophyll to peanut oil

were also significantly affected by the addition of chlorophyll to the soybean oil. However, several compounds that were significantly affected by chlorophyll in soybean oil were not detected in peanut oil. These compounds may be derived from linolenic acid, which is not found in peanut oil.

Hexanal

Hexanal was initially present in the peanut oil and accounted for approximately 10% of the total volatiles throughout the storage. The addition of chlorophyll to the peanut oil did not result in increases in the amount of hexanal in the oil compared to the 0 ppm chlorophyll oil exposed to light, indicating that hexanal is not formed in significant amounts by photosensitized oxidation relative to triplet oxygen oxidation. This characteristic is similar to that observed in soybean oil. The amount of hexanal increased over time in a linear manner in the oil ($R^2 > 0.87$) during the 10 day storage under light at 40 °C (Figure 4.4). The increase in the hexanal peak area was approximately 150/hr. The peak area increase in soybean oil was approximately 4.5 times as large as that measured in the peanut oil.

2-Heptenal

2-Heptenal was initially present in the peanut oil with a peak area of 7800 and was greatest in quantity in the peanut oil that contained 10 ppm added chlorophyll and had been exposed to light for 10 days at 40 °C (Figure 4.4). After 1 day of storage the increase in the amount of 2-heptenal in the 10 ppm peanut oil was over 10 times the

amount present in the oil containing 0 ppm chlorophyll stored at 40 °C. After 10 days of storage at 40 °C, the increase of 2-heptenal in the 10 ppm peanut oil was approximately 3.5 times the amount in the 0 ppm oil. For comparison, the amount of hexanal increased by approximately 7 times over the 7 day storage at 40 °C. The discrepancy between the amounts of 2-heptenal and hexanal is similar, but not as great in the peanut oil as it was in the soybean oil.

The amount of 2-heptenal increased over time in a linear manner at 143/hr in the oil containing 0 ppm chlorophyll when stored under light at 40 °C ($R^2 = 0.96$). The peak area increase of 2-heptenal in soybean oil containing 0 ppm chlorophyll stored at 40 °C was approximately 3.5 times that measured in peanut oil (Table 3.2). The increase of 2-heptenal in the peanut oil containing 1 ppm or 10 ppm chlorophyll stored at 40 °C was 227/hr and 564/hr, respectively. The production rate of 2-heptenal was 4 to 5.5 times as great as that measured in the peanut oil.

4.5 Conclusions

The storage temperature, concentration of chlorophyll and exposure to light were significant factors in the production of total volatiles and the singlet oxygen product 2-heptenal. Chlorophyll concentration had no effect on the amount of the triplet oxygen autoxidation product hexanal. The amount of hexanal in peanut oil was dependant on storage temperature, light exposure and the duration of storage. Light exposure had a significant effect on the production of all volatile compounds in peanut oil stored in the light for 10 days at 4 or 40 °C. The presence of 10 chlorophyll in peanut oil significantly increased the production of over half of the volatile compounds in peanut oil stored at 40

°C for 10 days. Volatile compounds in peanut oil with or without added chlorophyll were qualitatively similar. These characteristics were similar to that observed in the soybean oil. However, several compounds that were significantly affected by chlorophyll in soybean oil were not identified in peanut oil. Such products may be derived from linolenic acid, which is not present in peanut oil. The effect of chlorophyll was less prominent in the peanut oil compared to the soybean oil, particularly at 4 °C.

The increase in the formation of total volatiles, hexanal and 2-heptenal in the soybean oil stored at 40 °C was approximately 4 to 4.5 times greater than the increases in peanut oil stored at 40 °C. This is likely due in part to the differences in fatty acid composition of peanut oil and soybean oil. Peanut oil contains almost twice as much linoleic acid as peanut oil. The substrate for hexanal and 2-heptenal is linoleic acid, which is almost twice as plentiful in soybean oil as in peanut oil. Furthermore, soybean oil contains linolenic acid, which is approximately 2 times as susceptible to autoxidation initiation as linoleic acid. These factors would be expected to contribute to an increased rate of oxidation and volatile compound formation in soybean oil relative to peanut oil.

It was expected that products of autoxidation would be more affected by the difference in fatty acid composition between peanut oil and soybean oil than would be the products of singlet oxygen oxidation. However, the products of both types of oxidation were similarly affected by the difference in fatty acid composition; total volatiles, hexanal and 2-heptenal were all produced at a rate 4 to 5 times greater in the soybean oil. These results indicate that fatty acid composition is also an important factor in photoxidation as well and that autoxidation is an important component of volatile production resulting from photosensitized reactions.

	Soybean	Peanut
Saturated Fatty Acids	12%	21%
Unsaturated Fatty Acids	88%	79%
Polyunsaturated Fatty Acids	60%	32%

Table 4.1. Fatty acid composition of soybean oil and peanut oil.

Compound Identification	Initial Amount	Storage at 4 °C				Storage at 40 °C			
		0 ppm Dark	0 ppm Light	1 ppm Light	10 ppm Light	Dark	0 ppm Light	1 ppm Light	10 ppm Light
Pentane*	1216	1498	**2560**	2027	3631	5561	7960	9800	**13256**
1-Hexene	2924	2951	**5108**	4852	6417	2335	**12710**	14616	**21318**
1-Penten-3-ol	2731	2848	3221	3127	3264	1840	**2841**	3003	3148
Pentanal*	2032	3257	**6287**	5552	7000	5835	**10565**	12229	**13307**
1-Pentanol	537	597	**1490**	1097	1479	1109	**3360**	4015	**4854**
Hexanal	7218	13510	**22143**	15776	21709	25935	**45143**	50901	47281
2-Hexenal	ND	ND	ND	ND	ND	1222	**2796**	3252	**3501**
Heptanal	2308	1569	**2272**	1912	2537	2624	4051	5160	**4944**
2-heptenal*	7816	8038	**30786**	26046	**89460**	7264	**44003**	**62937**	**135520**
1-Octen-3-ol	2252	3318	**5479**	4840	**12904**	3583	**12002**	**18504**	**37473**
2-Pentyl Furan	1139	915	1137	917	1237	1712	**3796**	4030	**4981**
2-Octenal*	ND	ND	**841**	727	1344	4141	**10616**	**13850**	**18093**
Undecane	ND	ND	ND	ND	ND	6684	**17132**	**21629**	15842
Nonanal	971	814	**2191**	2115	1948	7021	10564	12236	10314
Total Volatiles*	69555	73176	**193283**	191574	**320746**	197364	**363751**	**483306**	**625247**

* Compound was also significanlty affected by 10 ppm chlorophyll after 24 hours of storage at 4 and 40 °C
ND = indicates that the compounds was not detected at these conditions
Peak areas of compounds that are significantly different (0 ppm dark vs 0 ppm light) and (0 ppm light vs 1 ppm light or 10 ppm light) are shown in bold

Table 4.2. Average peak areas of volatile compounds in peanut oil containing 0 ppm, 1 ppm or 10 ppm chlorophyll after 10 days of storage in the dark or under light at 4 or 40 °C.

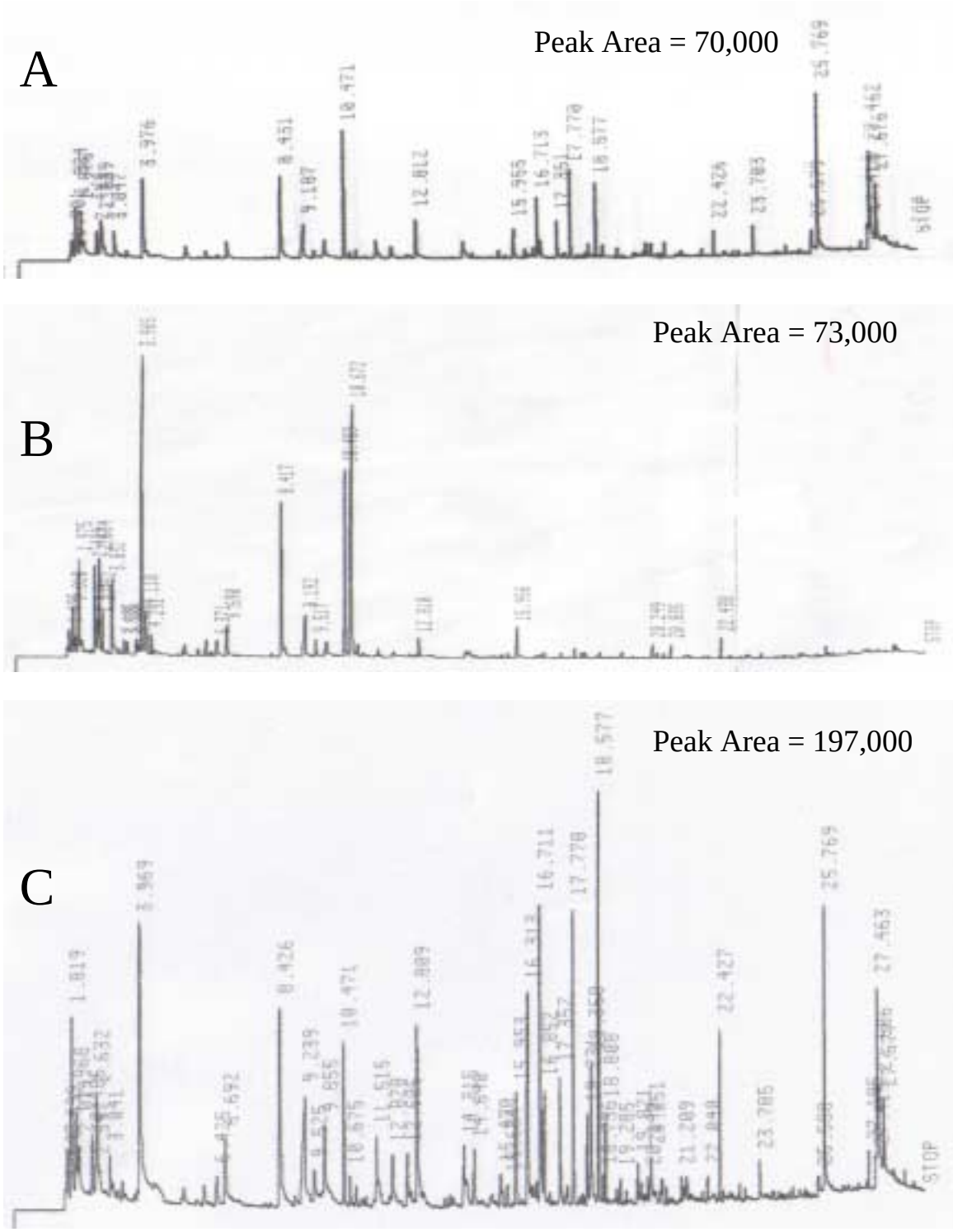


Figure 4.1. Volatile compounds in peanut oil (A) initially and after 10 days storage in the dark at (B) 4 °C and (C) 40 °C.

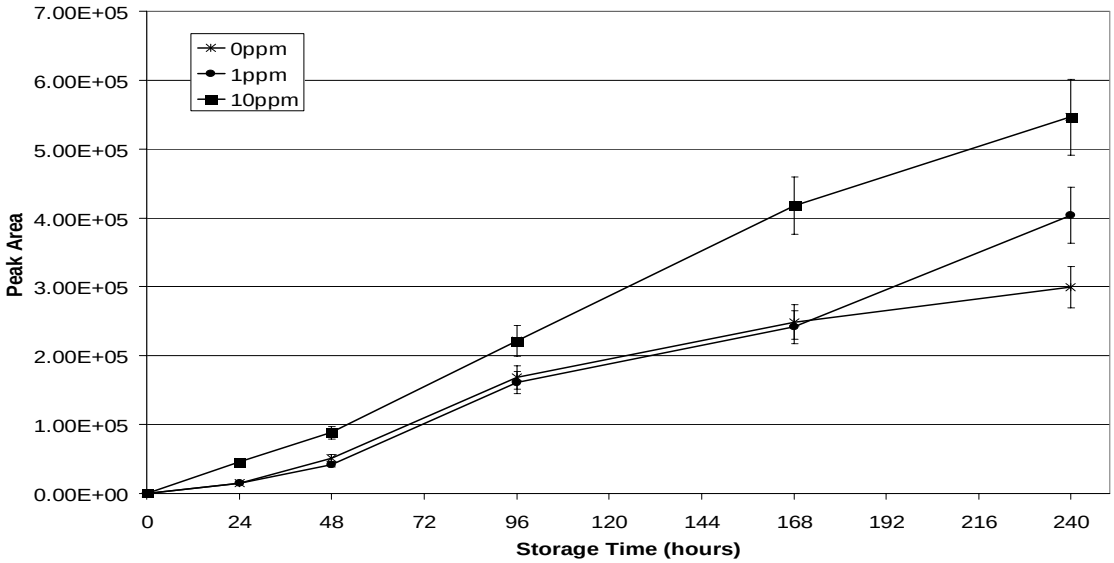


Figure 4.2. Total volatiles in peanut oil containing 0ppm, 1ppm or 10ppm chlorophyll exposed to light for 10 days stored at 40 ^{o}C.

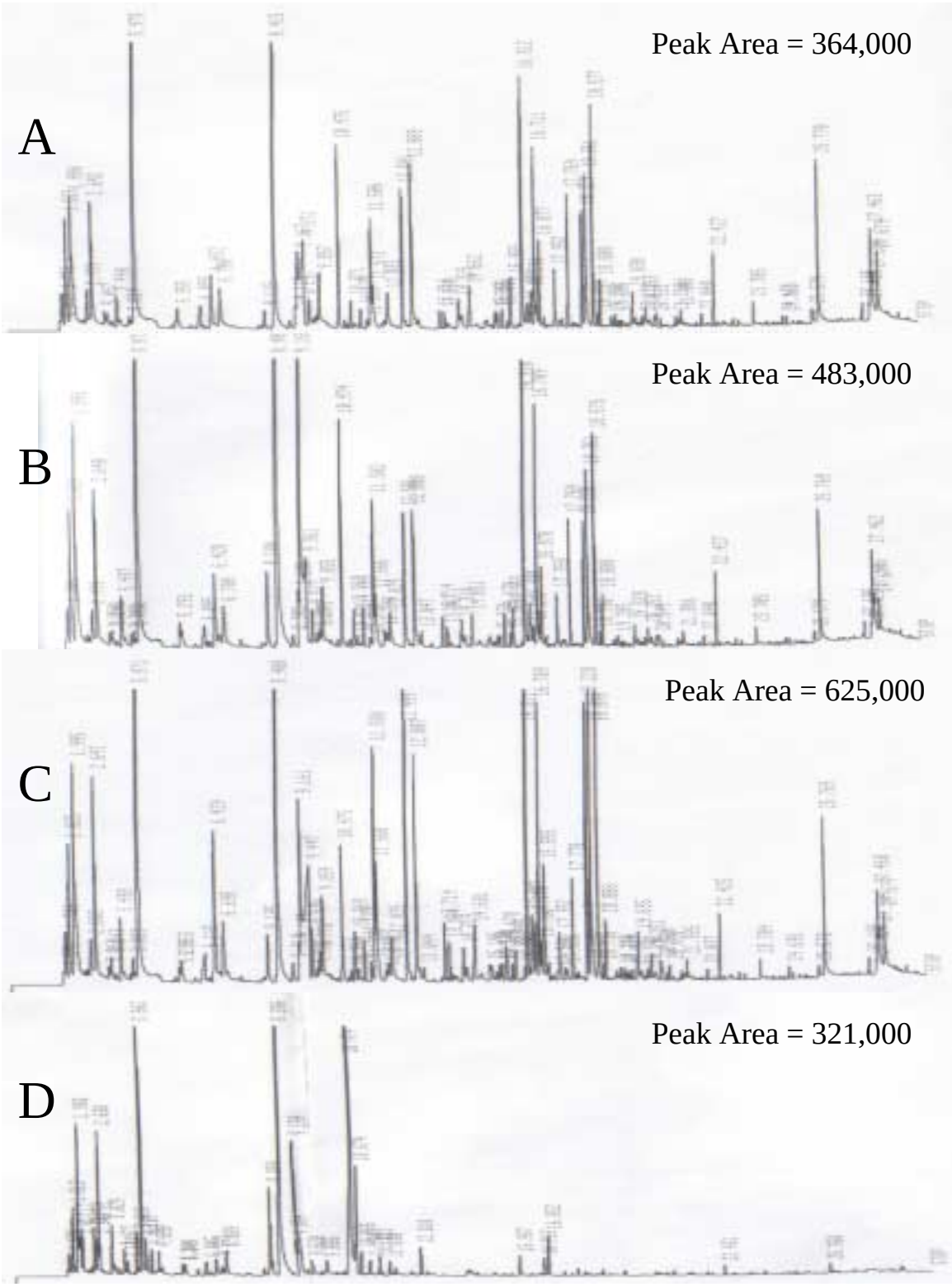


Figure 4.3. Volatile compounds in peanut oil exposed to light at 40 °C for 10 days containing (A) 0 ppm, (B) 1 ppm and (C) 10 ppm chlorophyll and exposed to light at 4 °C for 10 days containing 10 ppm chlorophyll (D).

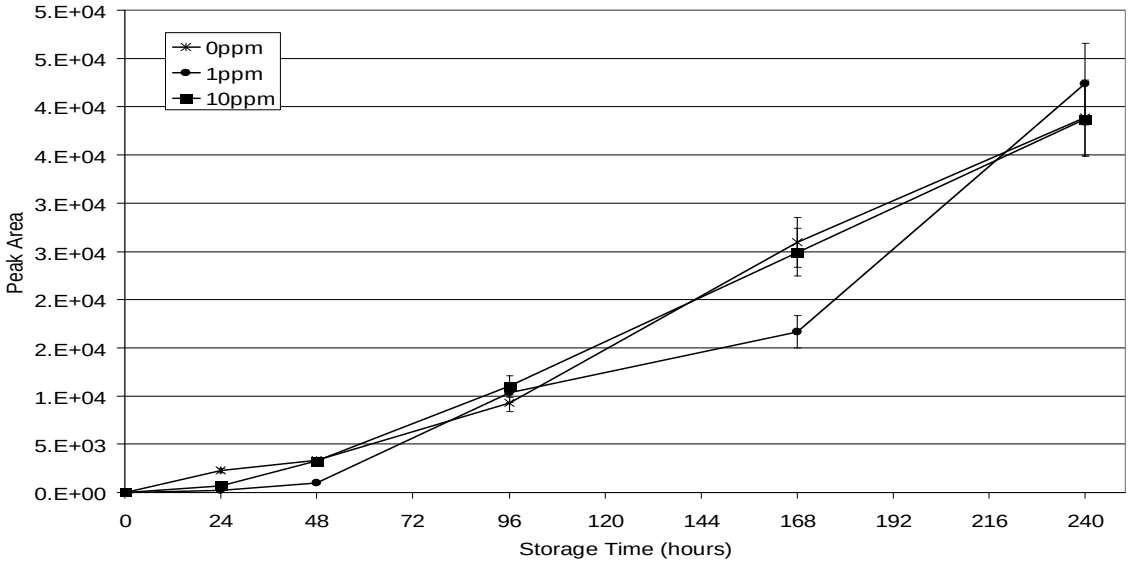


Figure 4.4. Hexanal in peanut oil containing 0ppm, 1ppm or 10ppm chlorophyll exposed to light for 10 days stored at 40 ^{o}C.

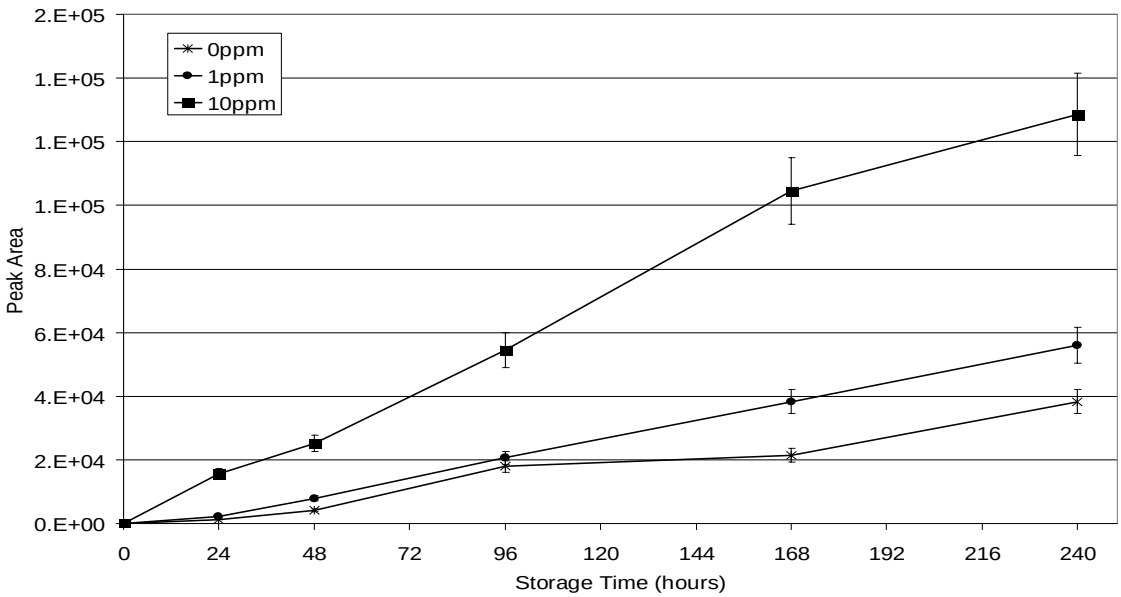


Figure 4.5. 2-Heptenal in peanut oil containing 0ppm, 1ppm or 10ppm chlorophyll exposed to light for 10 days stored at 40 ^{o}C.

CHAPTER 5

PHOTOXIDAITON AND PHOTOSENSITIZED OXIDATION OF LARD

5.1. Abstract

Lard naturally contains no photosensitizer and contains less unsaturated and polyunsaturated fatty acids than soybean oil and peanut oil. Based on the fatty acid composition and the results obtained from the previous two studies, it would be expected that the production of volatiles in lard would be less than that observed in soybean and peanut oil. The lard used in this study also contained the antioxidants BHA and BHT, which will inhibit autoxidation, but not singlet oxygen oxidation. The objectives of this research was to determine the effect of chlorophyll concentration on the production of volatiles in the lard compared to the results obtained from the soybean oil and peanut oil studies. Soybean oil containing 0, 1, 5 or 10 ppm chlorophyll was stored under light at 4, 20 and 40 °C for 7 days. Volatile compounds were analyzed by solid phase microextraction and gas chromatography.

Volatile compounds in lard with or without added chlorophyll were qualitatively similar. The presence of 1 ppm chlorophyll in lard significantly increased the production

of several volatile compounds in peanut oil exposed to light at 45 °C for 10 days, a characteristic not observed in either the peanut oil or the soybean oil. The antioxidants present in the lard can limit the autoxidation activity, thereby allowing the contribution of 1 ppm chlorophyll to the production of volatiles to be observed. Since 2-heptenal is primarily a singlet oxygen product, it was not as severely affected by the presence of antioxidants. Both autoxidation and singlet oxygen products were formed in lesser quantities in lard relative to soybean oil and peanut oil as expected due to compositional differences.

4.2. Introduction

Lard naturally contains no photosensitizer. Although myoglobin can act as a photosensitizer, it has low solubility in lipids and is not present in manufactured lard. Lard contains approximately 46% saturated fatty acids and 54% unsaturarted fatty acids. Approximately 40% of lard is composed of the saturated fatty acids palmitic and steric acid. Oleic acid accounts for about 45% of the lard. The polyunsaturated fatty acids linoleic and linolenic acid account for 9.5% and 0.5%, respectively. The oxidation of soybean oil, peanut oil and lard, containing different fatty acid compositions can be evaluated (Table 5.1). Based on the fatty acid composition and the results obtained from the previously two studies, it would be expected that the production of volatiles in lard would be less than that observed in soybean and peanut oil. The lard used in this study contained the antioxidants BHA and BHT. These antioxidants will inhibit autoxidation, but not singlet oxygen oxidation.

The primary objective of this research was to (1) determine the effect of chlorophyll concentration on the production of volatiles in lard, (2) determine the effect of fatty acid composition to the production of volatiles by comparison to the results obtained from the soybean oil and peanut oil and (3) determine if the presence of antioxidants in the lard have an affect on the production of volatile compounds.

4.3. Materials and Methods

Materials

Fischer's lard, produced and distributed by Fischer's Packing Co., Louisville, KY, was purchased from Giant Eagle Supermarket (Columbus, Ohio). Chlorophyll b and the chemical standards 1–pentenol, hexanal, 2-hexenal, heptanal, 2-heptenal, 1-octen-3-ol and 2,4-heptadienal were purchased from Sigma Chemical Co. (St.Louis, MO). The chemical standard 2-pentyl furan was purchased from Karl Industries Inc. (Aurora, OH). The gas chromatography (GC) columns, the polydimethylsiloxane/divinylbenzene (PDMS/DVB) solid phase microextraction fibers, fiber holder, glass GC inlet liners, 10ml glass serum vials, Teflon-coated rubber septa and aluminum crimp caps were purchased from Supelco, Inc. (Bellefonte, PA). Chlorophyll b and lard were stored at –20 °C until use. Other chemicals were stored at 4C.

Sample Preparation and Storage

Chlorophyll was added to the lard at concentrations of 1 or 10 ppm and mixed with a stirring bar for 4 hours at 50 ^{o}C in a sealed flask that had been flushed with nitrogen. A 2 ml aliquot of the prepared lard containing 0, 1, or 10 ppm added

chlorophyll was placed in a 10 ml clear glass vial and sealed with a Teflon-coated rubber septa and aluminum crimp cap in order to prevent contamination of the vial headspace. The lard was stored in a mirrored light box for 240 hours while exposed to a tungsten light (1560 lumens). The temperature in the light box was maintained at 45 °C ±2 °C throughout the duration of the storage. This temperature was necessary to maintain the lard in a liquefied state. A 2 ml aliquot of lard containing 0 ppm added chlorophyll was wrapped with foil to prevent light exposure and stored in the light box for 240 hours days at 45 °C ±2 °C. Prior research has established that chlorophyll has no effect on the production of volatile compounds if the oil is not exposed to light (Lee 2003); therefore, the oil stored in the dark did not contain chlorophyll.

Analysis of Headspace Volatile Compounds

Headspace volatiles were analyzed by solid phase microextraction (SPME) and gas chromatography/mass spectroscopy (GC/MS). Compounds were identified by the combination of retention times of standards and mass spectroscopy. Identification of compounds by mass spectroscopy MS was performed throughout storage.

Headspace volatile compounds were isolated by SPME with a 65 mm PDMS/DVB fiber. The fiber was inserted into the vial headspace for 30 minutes at 50 °C. SPME has been used for the extraction of volatile compounds from soybean and corn oil (Steenson et al 2002) and linseed and sunflower oil (Jalen et al 2000). Steenson and others (2002) reported that PDMS solid phase performed better than other solid phase materials for the extraction of volatile chemicals from soybean and corn oil. Jelen and others (2000) reported that solid phases containing DVB, carboxen and PDMS were

more sensitive to the extraction of volatile compounds from linseed and sunflower oil. Callison (2000) and Lee (2002) reported that PDMS/DVB was more sensitive than PDMS for the extraction of volatile compounds from soybean oil and linoleic acid.

Extracted volatiles were desorbed in the GC injection port for 5 minutes at 250 °C. The injection port was fitted with a 0.75 mm internal diameter splitless glass liner (Scheppers Wercinski 1999). Volatile compounds were separated and detected using an SPB-5 column (5% diphenyl / 95% dimethylsiloxane) 30 m x 0.25 mm x 0.25 mm film thickness (Supelco) in a flame ionization detector equipped Hewlett-Packard 6890 GC (Wilmington, DE) using helium carrier gas. The GC was programmed to hold at 60 °C for 5 minutes, increase to 120 at 4 °C/minute, increase to 240 at 10 °C/minute and hold at 240 °C for 2 minutes.

Statistic Analysis of Data

Three trials were run at each temperature. For each trail, headspace volatiles were collected and analyzed after 0, 1, 2, 4, 7, and 10 days. One way ANOVA, using Minitab, was used to determine if storage time, storage temperature and chlorophyll level were significant factors in the production of volatile compounds. Means of significant factors were compared using Tukey's studentized range ($\alpha = 0.05$). T-tests (two-tail, two-sample equal variance) were used for direct comparison of the amounts of each compound produced and calculated using Excel.

The effect of light on the production of volatile compounds was characterized by comparison of the lard containing 0 ppm chlorophyll that was stored in the dark to the lard containing 0 ppm chlorophyll that was exposed to light. The effect of chlorophyll on

the production of volatile compounds was characterized by comparison of the lard containing 0 ppm added chlorophyll to the lard that contains 1 ppm or 10 ppm added chlorophyll, all of which are stored under light.

4.4. Results and Discussion

Total Volatiles

The lard initially contained relatively few volatile compounds (Figure5.1). The amount of volatiles increased over time in the lard stored at 45 °C. Coefficients of variation for the amount of compounds produced in lard were typically 5% to 15%. ANOVA showed that light exposure, storage time and the amount of added chlorophyll were all significant factors ($p<0.05$) in the production of total volatiles in lard.

When the lard containing 0 ppm to 10 ppm chlorophyll was exposed to light, there was a significant increase in the amount of total volatiles compared to the amounts present in the lard stored in the dark (Figure 5.2). Unlike soybean oil and peanut oil, the amount of total volatiles increased over time in a non-linear manner when the lard was stored under light at 45 °C. The amount of volatiles in lard containing 0 ppm and 1 ppm chlorophyll changed little between 1 and 7 days of storage. The increase in peak area for total volatiles in lard containing 0 ppm, 1 ppm or 10 ppm chlorophyll after 10 days of storage was 77,000, 140,000 and 180,000, respectively. For comparison, the increase in peak area for total volatiles in peanut oil containing 0 ppm, 1 ppm or 10 ppm chlorophyll after 10 days of storage was 166,000, 286,000 and 429,000, respectively. Therefore, the increase in peanut oil was 2 to 2.5 times that in lard. This disparity may be a result of

both the presence of antioxidants in the lard and the lower amount of polyunsaturated fatty acids in the lard.

Light

T-tests for the amounts each compound in the lard containing 0 ppm chlorophyll and 1 ppm chlorophyll were calculated after 1, 2, 4, 7 and 10 days of storage in order to determine if the compound was significantly affected by light exposure. Most of the compounds analyzed (Table 5.1), were significantly affected by light exposure ($p<0.05$). These results are similar to that obtained in the soybean oil and peanut oil stored at 40 °C.

Chlorophyll

Pentanal, 2-hexanal, 2-heptenal, 2-octenal and nonanal were in significantly greater amounts in the lard containing either 1 ppm or 10 ppm chlorophyll (Table 5.1). This differs from previous findings, where the presence of 1 ppm chlorophyll was not a significant factor to the production of most compounds in soybean oil and peanut oil that was stored at 40 °C. In those oils, storage at 40 °C would result in significant autoxidation, which can conceal any increased production of volatiles due to the presence of 1 ppm added chlorophyll. However, the antioxidants present in the lard can limit the autoxidation activity, thereby allowing the effect of 1 ppm chlorophyll to be observed. As with the soybean oil and peanut oil, most of the compounds analyzed in lard were significantly affected by the presence of 10 ppm chlorophyll after 10 days of storage. The effect of chlorophyll concentration to the production of volatiles after 2 days of light

exposure is shown in the chromatograms (Figure 5.3). 2-Heptenal was particularly affected.

Hexanal

Hexanal was initially present in the lard and was one of the most abundant compounds present throughout the storage. The amount of hexanal increased over time in a linear manner ($R^2 > 0.73$) in the lard during the 10 day storage under light at 45 °C (Figure 5.4). The presence of 1 ppm or 10 ppm chlorophyll resulted in significantly greater amounts of hexanal after 1 days of light exposure, a characteristic not observed in the soybean oil or peanut oil. Hexanal can be produced by singlet oxygen. However, since it is a primary product of autoxidation, the amount contributed by the singlet oxygen pathway was not apparent in the peanut oil and soybean oil. The presence of antioxidants in the lard can limit the autoxidation production of hexanal after 1 day of storage, thereby allowing the contribution of singlet oxygen to the production of hexanal to be observed. After 1 day of light exposure, the amounts of hexanal in the lard containing 0, 1 or 10 ppm chlorophyll were statistically similar. The increase in the hexanal peak area was approximately 18/hr, a much slower rate than the rate of 400/hr and 150/hr measured in the soybean and peanut oils, respectively. However, lard contains 1/5 and 1/3 the amount of linoleic acid as soybean oil and peanut oil, respectively. This, coupled with the presence of antioxidants, adequately explains in the lower production rate in lard.

2-Heptenal

2-Heptenal was initially present in the lard, with a peak area of 9800 and was greatest in quantity in the lard that contained 10 ppm added chlorophyll and had been exposed to light for 10 days at 40 °C (Figure 5.5). The amount of 2-heptenal in the lard containing 0 ppm chlorophyll did not increase over the 10 day exposure to light, a characteristic not observed in the soybean oil or peanut oil. The presence of antioxidants may eliminate the contribution of autoxidation to the production of 2-heptenal. The amount of 2-heptenal increased over time in a linear manner at 227/hr and 564/hr in the lard containing 1 ppm or 10 ppm chlorophyll stored at 45 °C ($R^2 = 0.97$). The production rate of 2-heptenal in lard containing 1 ppm or 10 ppm chlorophyll was 2 and 3 times lower than the production rate in peanut oil. However, this corresponds to the compositional difference; peanut oil contains 3 times as much linoleic acid as lard. Since 2-heptenal is primarily formed by singlet oxygen oxidation of linoleic acid, the production of 2-heptenal in lard was not as severely reduced as was the production of hexanal.

4.5. Conclusions

Chlorophyll concentration and exposure to light were significant factors in the production of total volatiles and the singlet oxygen product 2-heptenal. Chlorophyll concentration had no effect on the amount of the triplet oxygen autoxidation product hexanal after 2 days of storage, but was a significant factor at 1 day of storage. Light exposure and the presence of 10 ppm chlorophyll had a significant effect on the production of most volatile compounds in lard stored in the light for 10 days at 45 °C.

Volatile compounds in lard with or without added chlorophyll were qualitatively similar. These characteristics were similar to that observed in the peanut oil and soybean oil.

The presence of 1 ppm chlorophyll in lard significantly increased the production of several volatile compounds in peanut oil exposed to light at 45 °C for 10 days, a characteristic not observed in either the peanut oil or the soybean oil. The antioxidants present in the lard can limit the autoxidation activity, thereby allowing the contribution of 1 ppm chlorophyll to the production of volatiles to be observed. Since 2-heptenal is primarily a singlet oxygen product, it was not as severely affected by the presence of antioxidants. Both autoxidation and singlet oxygen products were formed in lesser quantities in lard relative to soybean oil and peanut oil as expected due to compositional differences.

	Soybean	Peanut	Lard
Saturated Fatty Acids	12%	21%	46%
Unsaturated Fatty Acids	88%	79%	54%
Polyunsaturated Fatty Acids	60%	32%	10%

Table 5.1. Fatty acid composition of soybean oil, peanut oil and lard

Compound Identification	Initial Amount	Storage at 45 °C			
		Dark Dark	0 ppm Light	1 ppm Light	10 ppm Light
Pentane*	5885	3581	**6376**	6467	**9038**
1-Penten-3-ol*	ND	2186	**3545**	3792	3533
Pentanal*	ND	3224	**6392**	**10111**	**11588**
1-Pentanol	3195	2837	**3663**	3661	**4127**
Hexanal*	11784	11482	**17711**	**19215**	17994
2-Octene	ND	1214	1141	1218	**2070**
2-Hexenal	ND	3681	2879	**4704**	**4497**
Heptanal	3494	3604	**4966**	4976	**3972**
2-heptenal*	9803	9499	**10447**	**25731**	**78758**
1-Octen-3-ol*	6452	9359	**7545**	8102	**19488**
2-Pentyl Furan	ND	1599	**3115**	3171	**3838**
2-Octenal	5119	5124	**5675**	**6959**	**8073**
Undecane	ND	1137	**2597**	1819	2919
Nonanal	4185	4762	**6735**	**8097**	**7768**
Total Volatiles	240737	284202	**317451**	**380539**	**420789**

* Compound was also significanlty affected by either 1ppm or 10ppm chlorophyll after 24 hours at 45 °C

ND = indicates that the compounds was not detected at these conditions

Peak areas of compounds that are significantly different (0 ppm dark vs 0 ppm light) and (0 ppm light vs 1 ppm light or 10 ppm light) are shown in bold

Table 5.2. Average peak areas of volatile compounds in peanut oil containing 0 ppm, 1 ppm or 10 ppm chlorophyll after 10 days of storage in the dark or under light at 45 °C.

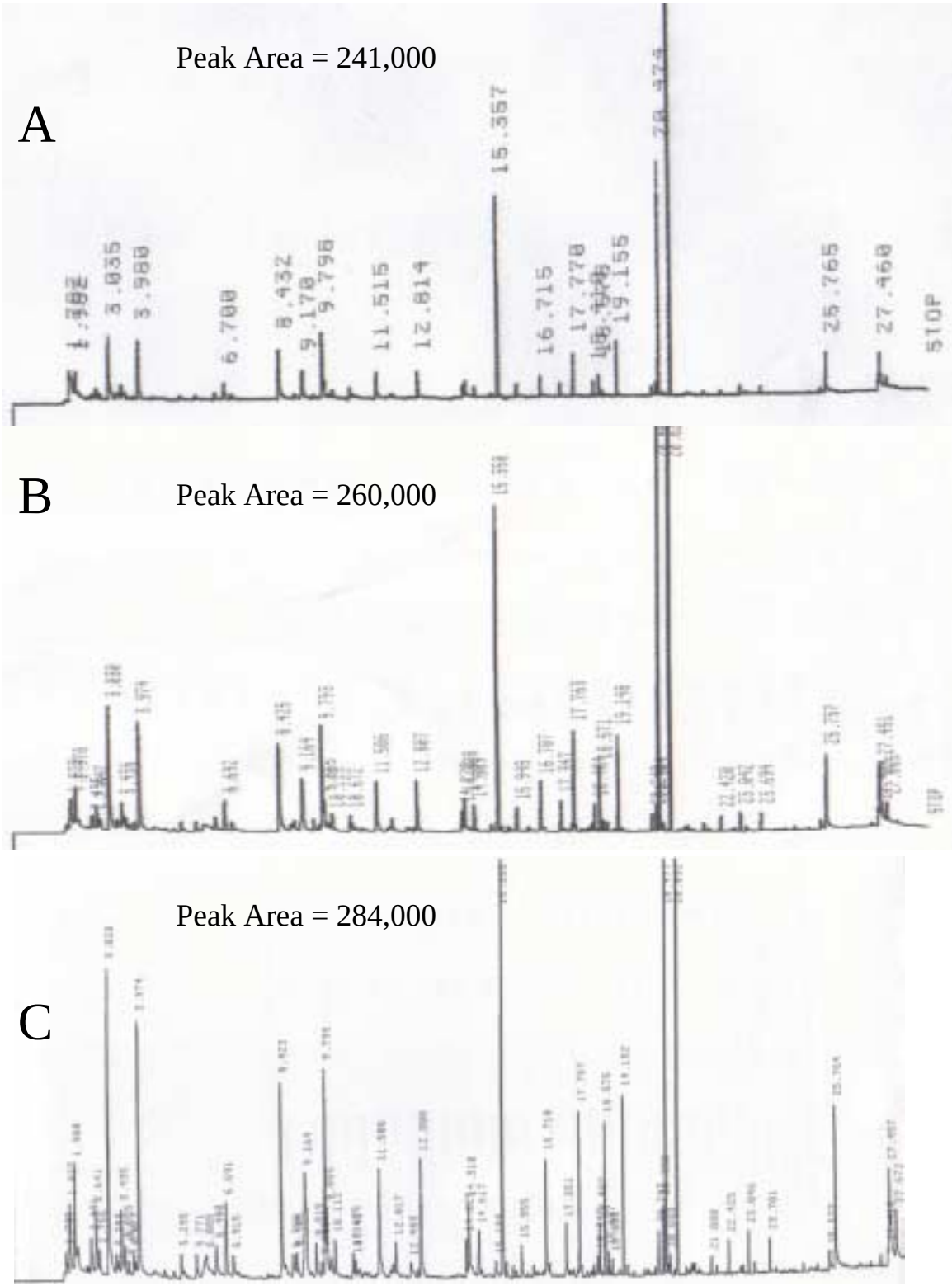


Figure 5.1. Volatile compounds in lard stored in the dark at 45 ºC after (A) 0 days, (B) 2 days and (C) 10 days.

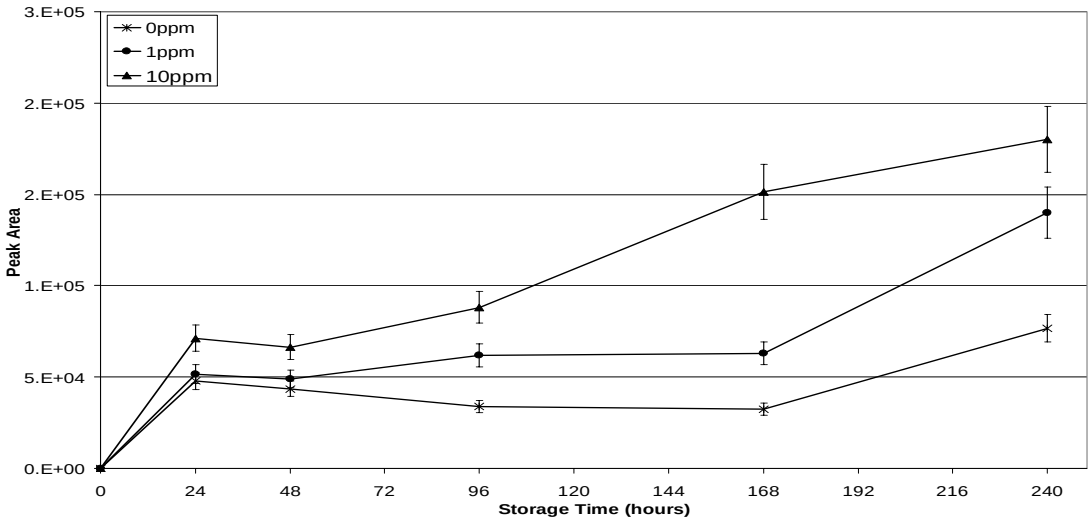


Figure 5.2. Total volatiles in lard exposed to light at 45 ºC containing 0ppm, 1ppm or 10ppm chlorophyll.

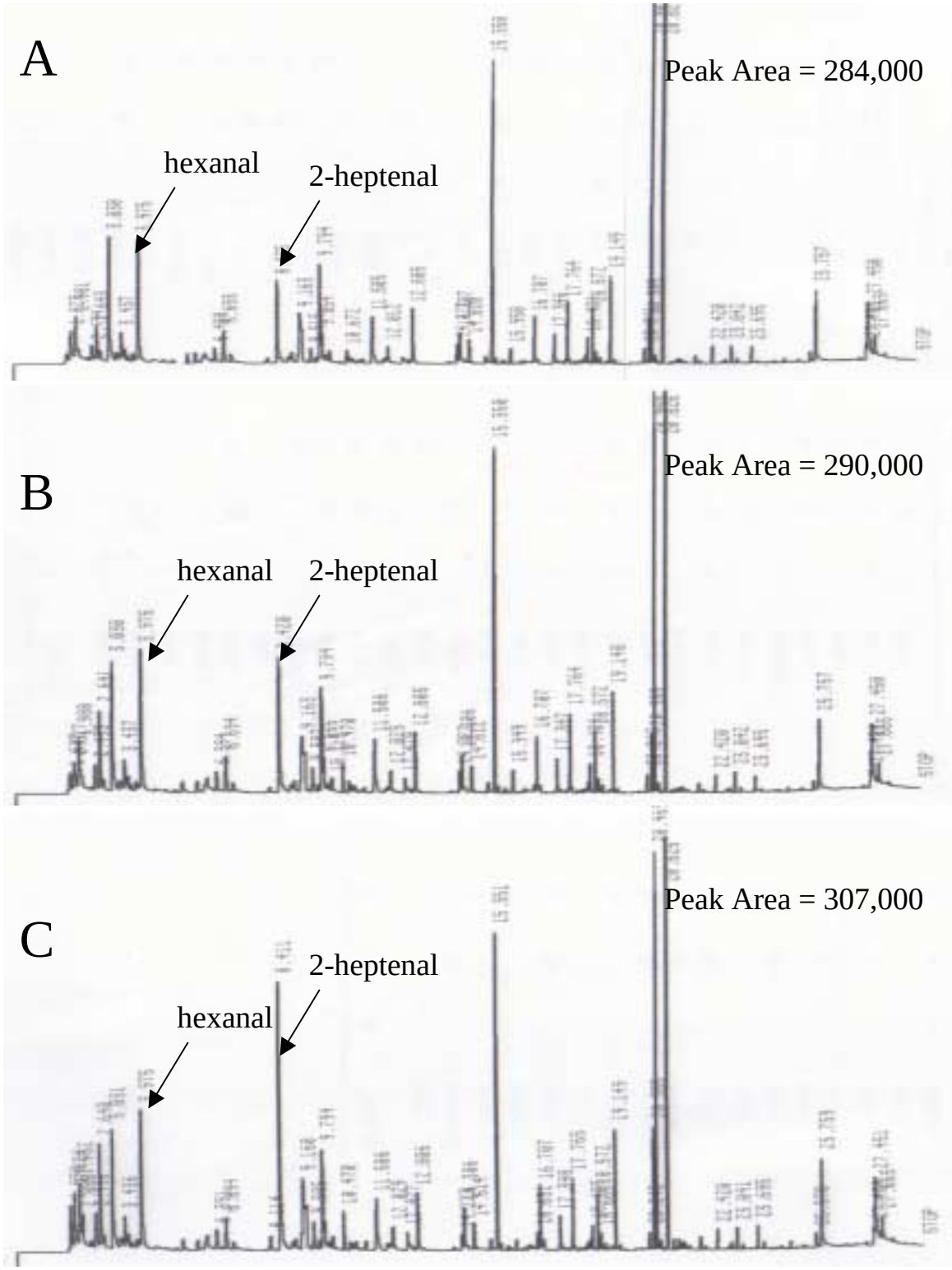


Figure 5.3. Volatile compounds in lard exposed to light for 2 days containing (A) 0 ppm, (B) 1 ppm and (C) 10 ppm chlorophyll.

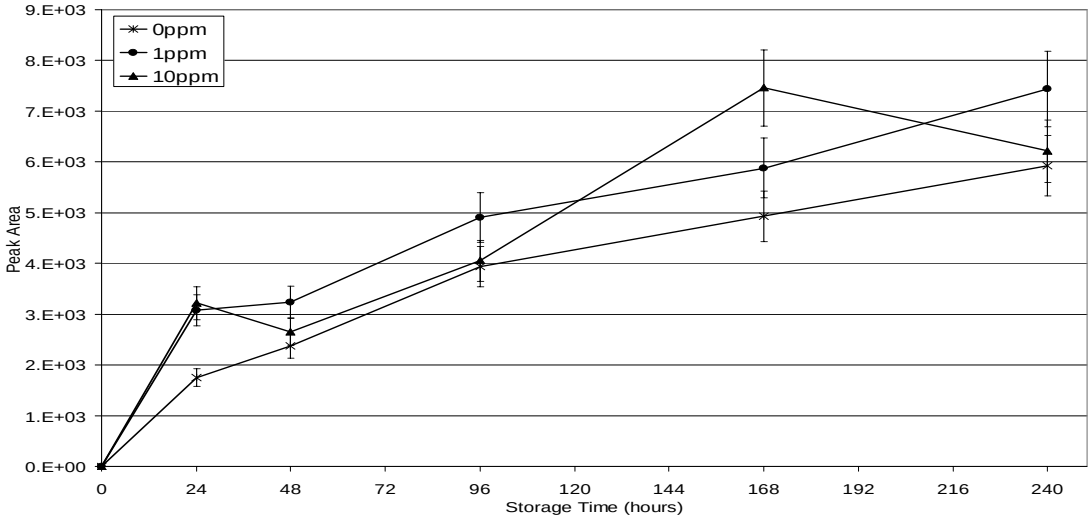


Figure 5.4. Hexanal in lard containing 0ppm, 1ppm or 10ppm chlorophyll exposed to light at 45 ^{o}C.

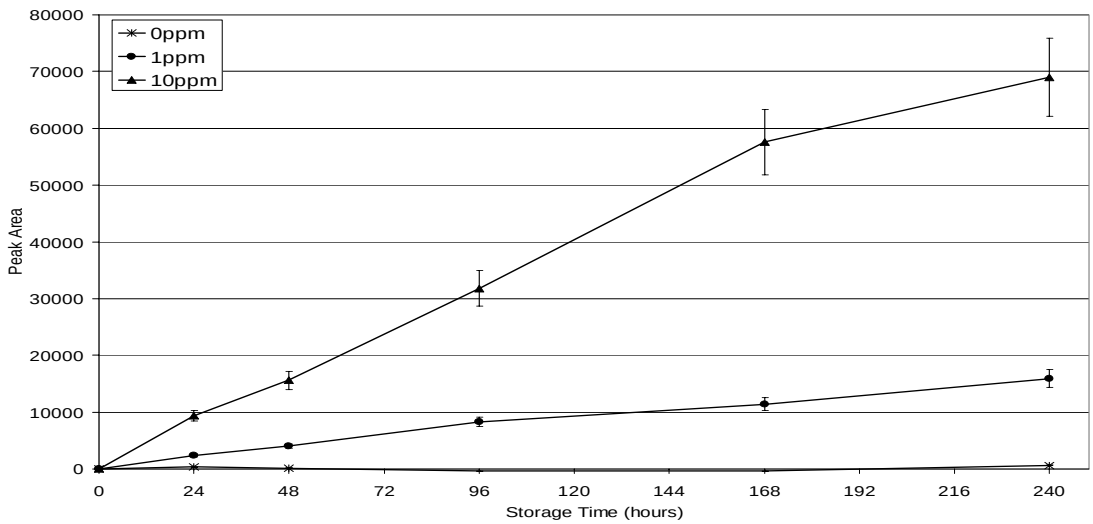


Figure 5.5. Amount of 2-heptenal in lard containing 0ppm, 1ppm or 10ppm chlorophyll exposed to light at 45 ^{o}C.

CHAPTER 6

CARBON DIOXIDE IN HIGH PRESSURE PROCESSING: ENZYMATIC AND CHEMICAL CHANGES IN SINGLE STRENGTH ORANGE JUICE

6.1. Abstract

High-pressure processing (HPP) in combination with added $CO_{2(g)}$ was examined for efficacy in inactivating Valencia orange juice pectinmethylesterase (PME). Post-process chemical changes in ascorbic acid and headspace volatiles were evaluated in HPP treated samples, with and without added $CO_{2(g)}$. Pressure magnitude, temperature, and dwell time were significant factors ($p < 0.001$) in the inactivation of PME. Gas concentration increased the rate of PME inactivation ($p < 0.001$) across all of levels of pressure, temperature, and time. $CO_{2(g)}$ addition can enhance PME inactivation beyond that achieved by high pressure alone. Pressure treatments resulted in insignificant volatile losses and minor ascorbic acid reduction. Gas treatments resulted in significant volatile losses.

6.2. Introduction

Orange juice is the most predominant fruit juice in the U.S market today. Over 90% of the Florida orange crop is processed into juice or concentrate (Baker and Cameron 1999). Commercial orange juice is heat pasteurized to inactivate pectinmethylesterase (PME) and to inactivate spoilage organisms. It is necessary to inactivate PME in order to produce an acceptable orange juice with a prolonged shelf life; however, the heating process degrades the organoleptic and nutritional qualities of the juice (Reynolds 1963, Klavons and others 1994, Braddock 1999). Orange PME is bound to the cell walls and is released upon extraction (Rombouts and others 1982; Van den Broeck and others 1999). PME hydrolyses the C-6 methyl esters of polygalacturonic acid residues in pectin to yield methanol and pectic acid (Cameron and others 1998). Divalent cations, such as calcium, can then cross-link pectic acid units to adjacent pectin chains, thereby reducing their solubility resulting in cloud loss (Wicker and Temelli 1988; Baker and Cameron 1999). By industrial standards, enzymatic clarification of the cloud in citrus juice is considered a product quality defect (Braddock 1999).

High pressure food processing (HPP) can inactivate the thermal-labile PME isozyme (TL-PME), and can also inactivate spoilage microorganisms, such as lactic acid bacteria, yeast, and mold (Ogawa and others 1990; Basak and Ramaswamy 1998; Goodner and others 1998; Nienaber and Shellhammer 2001). Van den Broeck and others (1999) demonstrated that the use of additives could enhance PME inactivation by lowering the pH, or reducing calcium concentration in the juice. Published data has indicated that added carbon dioxide can lower the pH in orange juice (Balaban and others 1991; Meyssami and others 1992). Balaban and others (1991) have effectively

demonstrated that the use of supercritical carbon dioxide can inactivate PME and render a stable cloud in orange juice without the use of extremely high pressures.

Most of the flavor of orange juice is derived from its volatile components (Steffen and Pawliszyn 1996). Numerous studies have identified and assessed the significance of these volatile chemicals to the flavor of orange juice (Shaw and others 1993; Jia and others 1998; Farnworth and others 2001). The flavor quality of orange juice can be assessed by qualitative and quantitative analysis of volatile chemicals. Thermal processing and handling of orange juice can be detrimental to its chemical constituents such as flavor volatiles and ascorbic acid (Braddock and others 1999). While $CO_{2(g)}$ assisted HPP of orange juice has been shown to have positive effects on PME inactivation and microbial stability (Corwin and Shellhammer 2000), its effects on the flavor and ascorbic acid content have yet to be assessed. The use of $CO_{2(g)}$ in HPP may significantly reduce treatment pressure and processing times, thereby improving HPP cost efficiency and overall product quality particularly in regards to volatiles and ascorbic acid. The objectives of this study were to determine the effects of high pressure in combination with $CO_{2(g)}$ on the pressure magnitude, temperature, and dwell time required for inactivating PME of fresh Valencia orange juice, and to evaluate chemical changes in ascorbic acid and headspace volatiles.

6.3. Materials and Methods

Juice sample preparation

Freshly squeezed, unpasteurized, single strength Valencia orange juice was prepared in a commercial citrus pilot plant in Florida. The juice was rapidly frozen, and shipped overnight to Columbus, Ohio. The juice was stored at –25 °C until use. Prior to use, it was thawed in an ambient running water bath at 25 °C for 8 hours, and then held at 4 °C overnight. The juice was filtered with a coarse stainless steel strainer (2 mm) to ensure an even distribution of pulp level in all juice treatments, and to facilitate the bottling process of the carbonated juice. Acid adjustment experiments were performed using 5N citric acid (Fisher Scientific, Pittsburgh, PA).

CO_2 gassing apparatus

The orange juice was equilibrated with the assigned gas pressure in a 3-gallon stainless steel mini-keg vessel (N.S.D.A-V.S–0, Spartanburg Steel Products, Inc., Spartanburg, SC) at approximately 0 °C in an ice water bath for 30 min with periodic agitation to facilitate gas transfer. The carbonated juice was then filled under pressure into 30 ml wide mouth low density polyethylene (LDPE) bottles (Fisher Scientific, Pittsburgh, PA) using the Zahm and Nagel Model 9000-R hand filler (Zahm & Nagel, Inc., Buffalo, NY). After bottling, the juice was transferred to a high-pressure vessel and processed according to the experimental design. Following high pressure treatment, $CO_{2(g)}$ gas pressure (psig) measurements were made at 0.3 ± 0.26°C using the Zahm Model D.T. Piercing Device (Series 6000, Zahm & Nagel, Inc., Buffalo, NY). Liberation of $CO_{2(g)}$ took place in a

Crest de-gassing apparatus (Crest Ultrasonics, Corp., Trenton, NJ) for 10 minutes at room temperature for the volatile and ascorbic acid tests and for 3 minutes at room temperature for the remainder of the tests. The time between bottling, pressure treatment and degassing was less than 2 hours.

High-pressure processing

Pressure treatment was performed using an ABB Quintus Food Processor QFP-6 Cold Isostatic Press (ABB Autoclave Systems, Columbus, OH). The pressure fluid was a mixture of distilled water and Houghto-Safe 620-TY, containing glycol derivatives, in a 1:1 ratio (Houghton International, Valley Forge, PA). Juice samples bottled in LDPE bottles were pre-chilled in the glycol mixture, and pressurization was initiated at specific temperatures necessary to compensate for adiabatic heating during pressurization. The pressure, product temperature, and external water-jacket temperature were monitored and recorded in 3 sec intervals using a 21X Micrologger (Campbell Scientific Inc., Logan, Utah) connected to a computer running PC208W datalogger support software (Campbell Scientific Inc., Logan, UT). Operating pressures and temperatures varied by no more than 0.58% and 8.2%, respectively, throughout each run.

Pectinmethylesterase assay

PME activity of degassed juice was determined titrimetrically at pH 7.7 in an agitated water bath at constant temperature, 30 °C, using the method of Rouse and Atkins (1955). In the initial phase of the assay, the addition of 2.0N NaOH was used to slowly raise the pH of the juice to pH 7.7. The pH was maintained using a Computer-Aided Titrimeter

(CAT) titration system (Fisher Scientific, Pittsburgh, PA) by addition of 0.05N NaOH from an automatic buret. The reaction mixture consisted of 10 ml juice and 40 ml of 1% (10 g/L) pectin (Sigma, St. Louis, MO) solution containing 0.1M NaCl (Fisher Scientific, Pittsburgh, PA). Each sample was analyzed in duplicate. Analysis time varied from 5 to 20 minutes depending on the level of PME activity. PME activity was calculated by the following formula:

$$\text{PME units/ml} = \frac{(\text{mL NaOH})\ (0.05\ \text{N NaOH})\ (1000)}{(10\ \text{mL sample})\ (\text{time})}$$

Volatile analysis

Volatiles were collected and analyzed by headspace solid-phase microextraction gas chromatography (SPME-GC) using a procedure modified from Jia and others (1998). Ethyl butyrate, α-pinene, myrcene, limonene, linalool, decanal and valencene) along with total volatiles were quantified (Figure 6.1). Comparison of retention times of standards (Aldrich Chemical Co., Milwaukee, WI) as well as mass spectroscopy was used to identify the aforementioned chemicals. A 2 ml aliquot of the treated orange juice was placed in a 10 ml glass vial and sealed with a Teflon coated rubber septa and aluminum crimp cap (Supelco, Inc., Bellefonte, PA). Volatile compounds were extracted via SPME using a 100 μm polydimethylsiloxane (PDMS) coated fiber (Supelco) by manual insertion into the vial headspace for 30 minutes at 40°C. Prior to use, the SPME fiber was conditioned for 60 minutes at 250°C according to manufacturer's specifications. Extracted volatiles were desorbed in the GC injection port for 5 minutes at 250°C. The

injection port was fitted with a 0.75 mm internal diameter splitless glass liner (Supelco). Volatile compounds were separated and detected using an HP-5 column (crosslinked 5% PH ME Siloxane) 30m x 0.32mm x 0.25μm film thickness (Supelco) in a flame ionization detector equipped Hewlett-Packard 5890 GC (Wilmington, DE) using helium carrier gas. The GC oven was held at 60 °C for 2 minutes, increased at 6°C /minute to 150, increased at 10°C /minute to 220°C and held for 5 minutes at 220°C. Peak area was calculated using a Hewlett-Packard HP 3396A integrator (Wilmington, DE). Samples were prepared according to experimental design, and each sample was analyzed in duplicate. Averages of duplicate measurements were used in statistical analysis.

Ascorbic acid analysis

Ascorbic acid was measured by HPLC using a procedure from Yeom and others (2001). A sample of the treated orange juice was placed in a micro centrifuge tube and centrifuged for 10 min at 10,000 rpm. The supernatant was filtered through a 0.45 μm disk filter and then measured by high-pressure liquid chromatography (HPLC). Ascorbic acid was detected using a Hewlett-Packard 1050 liquid chromatograph equipped with an autosampler, a C-18 column (5 μm particle size, 4.6 mm diameter, 250 mm length) with a C-18 guard column, and a UV detector set at 254 nm. The mobile phase was a 10:90 (v/v) mixture of methanol and water. The water was acidified to pH 2.8 using phosphoric acid (0.01%, v/v). The mobile phase was filtered with a 0.45 μm membrane filter and degassed using helium. Peak area was calculated using a Hewlett-Packard HP 3396A integrator (Wilmington, DE). Samples were prepared according to experimental design,

and each sample was analyzed in duplicate. Averages of duplicate measurements were used in statistical analysis.

Statistical analyses and experimental design

For the PME inactivation trials, treatment conditions were replicated twice using full factorial designs with the factors being: treatment pressure, treatment time, treatment temperature, and added $CO_{2(g)}$. For each observation, duplicate runs of the PME assay were averaged and utilized as the measured unit. For ascorbic acid and volatile analysis, treatment conditions were replicated six times using a full factorial design with the factors being: pressure treatment (2 levels: atmospheric and 800 MPa) and carbon dioxide (4 levels: 0 psia $CO_{2(g)}$, atmospheric or 14.7 psia $CO_{2(g)}$, 24 ± 2 psia $CO_{2(g)}$, 31 ± 2 psia $CO_{2(g)}$). Averages of duplicate measurements were used for statistical analyses. SAS (SAS system for Windows, release 7, SAS Institute, Cary, NC) was used for analysis of variance (PROC GLM) and correlation (PROC CORR) and regression (PROC REG) analyses. Means of significant factors were compared using Tukey's studentized range ($\alpha = 0.05$). Nonlinear regression of time-dependent data was performed using and Microsoft Excel.

6.4. Results and Discussion

To study the effects of carbon dioxide gas in assisting the inactivation of PME via high pressure, non-carbonated juice treatments and carbonated juice treatments were subjected

to a range of conditions from 200-600 MPa, 30-300 sec dwell time at pressure, and 15-50 °C at final processing temperature.

Effect of pressure on PME activity

Pressure treatments below 400 MPa have little effect on the inactivation of orange juice PME (Cano and others 1997; Basak and Ramaswamy 1998; Nienaber and Shellhammer 2001). To determine whether the addition of carbon dioxide would increase PME inactivation at these lower pressures, a full factorial experiment was performed at 200 and 400 MPa, 30 and 300 sec, and 15°C, with and without carbonation (Figure 6.2). The presence of $CO_{2(g)}$ (9.6 ± 1.4 psig $CO_{2(g)}$, or 0.213 ± 0.03 mol % CO_2) was highly significant ($p < 0.001$) at reducing PME levels, while pressure magnitude, time, and the two and three-way interactions (pressure×gas, pressure×time, time×gas, and pressure×gas×time) were insignificant. Although the presence of carbon dioxide produced a statistically significant reduction in PME activity, it did not reduce PME activity to a level that would warrant using very low pressures (<400 MPa).

A pressure threshold of 400 MPa was apparent, below which pressure did not affect PME activity and beyond which pressure had a significant effect on reducing PME activity ($p < 0.001$) (Figure 6.3). These results support similar findings elsewhere (Goodner and others 1998, Nienaber and Shellhammer 2001). Similar to the low-pressure experiments, $CO_{2(g)}$ concentration was a significant factor ($p < 0.05$) at reducing PME beyond that achieved by pressure alone. The pressure × $CO_{2(g)}$ interaction was insignificant. The greatest difference in PME reduction between the non-carbonated

juice and carbonated juice occurred at 600 MPa, 20.0 ± 1.75 °C, 5 min, where the non-carbonated juice had 64.2 ± 2.08% reduction, and carbonated juice (10.08 ± 0.52 psig) had 85.2 ± 1.72% reduction.

Temperature effects on PME activity

A temperature range of 20-50 °C was studied at the higher pressures (500-600 MPa) where pressure and gas effects were earlier observed to be significant (figures 4 & 5). Multiple linear regression was conducted using stepwise regression with an alpha of 0.1for entry and exit of significant factors to and from the model. Results from the regression analysis revealed that $CO_{2(g)}$, pressure, temperature (squared), and a three way gas × pressure × temperature interaction were highly significant ($p < 0.001$) in terms of PME reduction. The equation derived from regression analysis to characterize the behavior of PME inactivation under these conditions was:

$$\text{PME activity (units/ml)} = 8.27 - 0.135\cdot(\text{Gas}) - 0.011\cdot(\text{Pressure}) - 0.000533\cdot(\text{Temp})^2 + 0.00000442(\text{Gas}\cdot\text{Pressure}\cdot\text{Temp}) \quad (1)$$

Multicolinearity among significant factors was assessed by examining the correlation matrix of all factors. The three way interaction was moderately correlated with gas level ($r = 0.91$), while the correlation among all other significant factors was insignificant ($r < 0.32$).

The pressure and gas effects were again readily apparent. A comparison of Figure 6.4 with Figure 6.5 reveals the three-way interaction. That is, at 500 MPa, there was a

strong temperature dependency of PME reduction for carbonated and non-carbonated samples, and the pressure × $CO_{2(g)}$ interaction was absent. The presence of gas produced a proportional reduction in PME activity over pressure alone across all temperatures. However at 600 MPa, the pressure × $CO_{2(g)}$ interaction was apparent. The PME reduction was temperature dependent in the non-carbonated samples, while in the presence of $CO_{2(g)}$, PME reduction was independent of temperature. Previous studies have reported that increasing temperature with pressure improved PME inactivation (Balaban and others 1991; Van den Broeck and others 1999; Nienaber and Shellhamer 2001). In this study, the presence of carbon dioxide confounds the temperature effect by eliminating the temperature dependency at higher pressure levels.

Effect of dwell time on PME activity

The time-dependency of PME reduction for the carbonated and non-carbonated samples was determined by measuring PME activities following different processing time intervals under constant conditions. A three parameter exponential response (equation 2) was fitted to data from four independent experimental runs at 600 MPa, 25 °C using Microsoft Excel:

$$y = y_{\infty} + a \cdot e^{(-b \cdot t)} \quad (2)$$

where y = PME activity (units/ml), y_{∞} = remaining PME activity unaffected by processing treatments (units/ml), a = total reduction in activity, i.e. the difference in amount of activity between unprocessed juice and y_{∞} (units/ml), and b = inactivation rate (sec^{-1}). Parameter estimates produced r^2 values in excess of 0.99 in all cases. Parameter

estimates were averaged to yield the response in Figure 6.6 and were compared using analysis of variance. No significant differences were apparent in y_∞ or a , while the rate of decay (b) was significantly different ($p < 0.01$), between the carbonated (12.61 ± 1.15 psig) and non-carbonated juices, with decay constants of 1.55 ± 0.36 sec^{-1} and 0.41 ± 0.16 sec^{-1}, respectively. The non-carbonated juice showed a response that continued to decay beyond the 5 min length of processing. The presence of $CO_{2(g)}$ can significantly reduce the dwell time by as much as three fold when compared to the non-carbonated juice, which was processed under the same conditions. For example, to inactivate 85% of PME activity, or render 15% residual PME activity after processing, the time needed was estimated to be ~346 sec in the no-gas treatment, but only 111 sec in the carbonated juice with ~12 psig $CO_{2(g)}$. The remaining active PME following pressure treatment was likely the thermo-stable isozyme (Goodner and others 1998).

Effect of juice pH on PME pressure sensitivity

Prior studies have reported that reductions in juice pH enabled greater PME inactivation at lower temperature(s) and/or at lower pressure(s) (Balaban and others 1991; Meysasami and others 1992; Snir and others 1996; Van den Broeck and others 1999). Meysasami and others (1992) discovered that $CO_{2(g)}$ had a direct influence on pH when dissolved in solution. Balaban and others (1991) reported a drop of as much as 0.7 pH units in orange juice under supercritical CO_2 conditions, i.e. saturated at 34 MPa. Even though the conditions examined in this study were different from the conditions Balaban and others (1991) examined, a common hypothesis was recognized: dissolved $CO_{2(g)}$ could lower the

pH of the juice under extreme high pressures and lead to more rapid reduction in PME activity. While it was not possible to measure the pH of the carbonated juice at 600 MPa, experiments using acidified juices were carried out in an effort to mimic transient pH reductions under pressure. The pH of fresh juice (pH = 3.77) was lowered by 0.5 units (to pH = 3.27) and 1.0 unit (to pH = 2.77) through the addition of 5N citric acid and then pressure processed at 600 MPa, 25°C.

The PME inactivation rates of pressure treated acidified and control (natural pH) juices were analyzed as described earlier, i.e. non-linear regression using equation (2) followed by analysis of variance of equation parameters. Comparison with earlier $CO_{2(g)}$ trials was accomplished by normalizing all data to their respective controls. The extent of inactivation was measured as the fraction of active PME remaining relative to the unprocessed juice (Figure 6.7). Differences in y_∞ and a were significant ($p<0.05$) only between the control juice and the highly acidified juice (pH 2.77), indicating that mild acidification and $CO_{2(g)}$ treatments had similar effects on the total reduction and percent remaining PME activity. Differences in inactivation rates were highly significant with two groups apparent. The acidified juices, though not statistically different from each other, had significantly greater PME inactivation rates (b) than the control and carbonated juices. It is likely that carbonating juice produced a temporary but not dramatic reduction in juice pH, and this reduction might be further augmented by the application of high hydrostatic pressure. Juice pH was determined to be the same prior to and following the gassing, pressurization, and degassing steps. The temporary effects of carbonating

orange juice did not affect the total percentage of PME inactivation but affected the rate at which the inactivation occurred when compared to the non-carbonated control.

Effect of gas and pressure treatments on headspace volatiles and ascorbic acid

To determine the effect of gassing, pressurizing, and degassing fresh orange juice on changes to volatile aroma and ascorbic acid content, samples were carbonated according to the experimental design and pressure treated at 800 MPa, 30 °C for 5 min. Analysis of variance of the total peak areas showed that loss of volatiles was dependent on added $CO_{2(g)}$ but not on pressure application (Table 6.1). The pressure × gas interaction was also insignificant. Loss of total volatiles was significantly correlated with added $CO_{2(g)}$ ($r = -.62$, $p<0.0001$) (Figure 6.8), ranging from an 11.0% loss at 14 psig $CO_{2(g)}$ to a 22.4% loss at 31 psig $CO_{2(g)}$. Average loss of volatiles across all gas levels was 15.4%. The losses in volatiles were believed to occur during $CO_{2(g)}$ addition and removal, when the $CO_{2(g)}$ can strip volatiles away from the juice. Supporting this theory was the fact that the compounds ethyl butyrate, α-pinene and myrcene, having short retention times, showed a greater loss due to added $CO_{2(g)}$ (Table 6.1), and greater negative correlations with gas level than those compound with longer retention times. The short retention compounds tend to be more volatile and would be expected to show greater losses corresponding to added $CO_{2(g)}$ during gas transfer operations, if stripping had occurred. The amount of α-pinene in the 31 psig $CO_{2(g)}$ samples was 31.9% less than that of the control whereas the valancene measured in the 31 psig $CO_{2(g)}$ sample was only 14.4% lower than the control. The $CO_{2(g)}$ treatment had no effect on the ascorbic acid retention.

Pressure contributed less to volatile losses than gas effects, however the contrary was evident for ascorbic acid losses. Of the identified volatiles, only mycerene showed a statistically significant loss, 15.7% ($p < 0.001$) resulting from the pressure treatment. The amount of measured ethyl butyrate increased as a result of the pressure treatment. HPP resulted in a reduction of ascorbic acid by 7.5% ($p < 0.05$) across all gas treatments. This modest reduction may be due to a combination of handling operations, which could have resulted in increased exposure to oxygen and the slight increase in temperature during the HPP operations. While the ascorbic acid losses were statistically significant, they were not large. Prior studies have shown that pasteurization had an insignificant effect on the loss of ascorbic acid (Sadler and others 1992; Yeom and others 2000).

6.5. Conclusions

Pressure processing does not significantly alter the volatile profile of fresh, single strength orange juice, except for mycerene and ethyl butyrate, but does result in minor reductions in ascorbic acid. The addition of carbon dioxide to the process significantly affects orange juice volatiles, and this phenomenon is hypothesized to be due to the method of gassing and degassing the juice. Combining carbon dioxide with high pressure processing of fresh orange juice can significantly increase the rate of PME inactivation beyond that achieved by pressure alone. The implication of this finding is potentially shorter dwell times at reduced pressures. The total degree of PME inactivation and/or the fraction that remains active following treatment is unchanged by the addition of $CO_{2(g)}$ to the process.

Table 6.1. HPP and added carbon dioxide affects on the ascorbic acid content and headspace volatiles of treated orange juice. Processed at 800 MPa, 30 ^{o}C and 0 psig $CO_{2(g)}$, 14 psig $CO_{2(g)}$, 24 ± 2 psig $CO_{2(g)}$, 31 ± 2 psig $CO_{2(g)}$), n=6

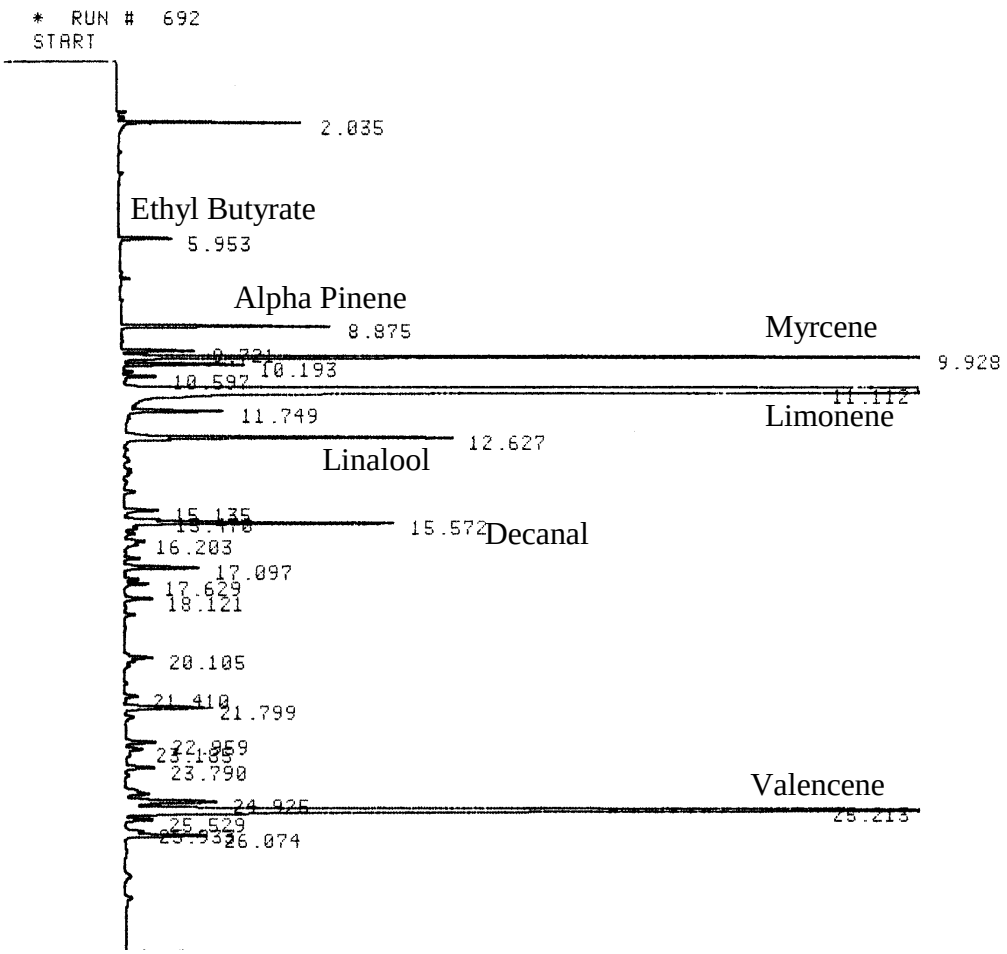
	Pressure[1] Treatment	% Change	Gas[2] Treatment	% Change
Ethyl Butyrate	***	28.1%	***	-18.0%
alpha Pinene	NS		***	-25.2%
Myrcene	***	-15.7%	**	-16.5%
Limonene	NS		***	-19.0%
Linalool	NS		*	-2.5%
Decanal	NS		**	-12.0%
Valencene	NS		NS	
Total Volatiles	NS		***	-15.4%

(NS) Not Significnat, (*) p<0.05, (**) p<0.01, (***) p<0.001

1 Significance of HPP on the retention of volatiles (all levels of CO_2)

2 Significance of added CO_2 on the retention of volatiles (all levels of CO_2)

Figure 6.1. Identified peaks of orange juice by headspace SPME-GC-MS.



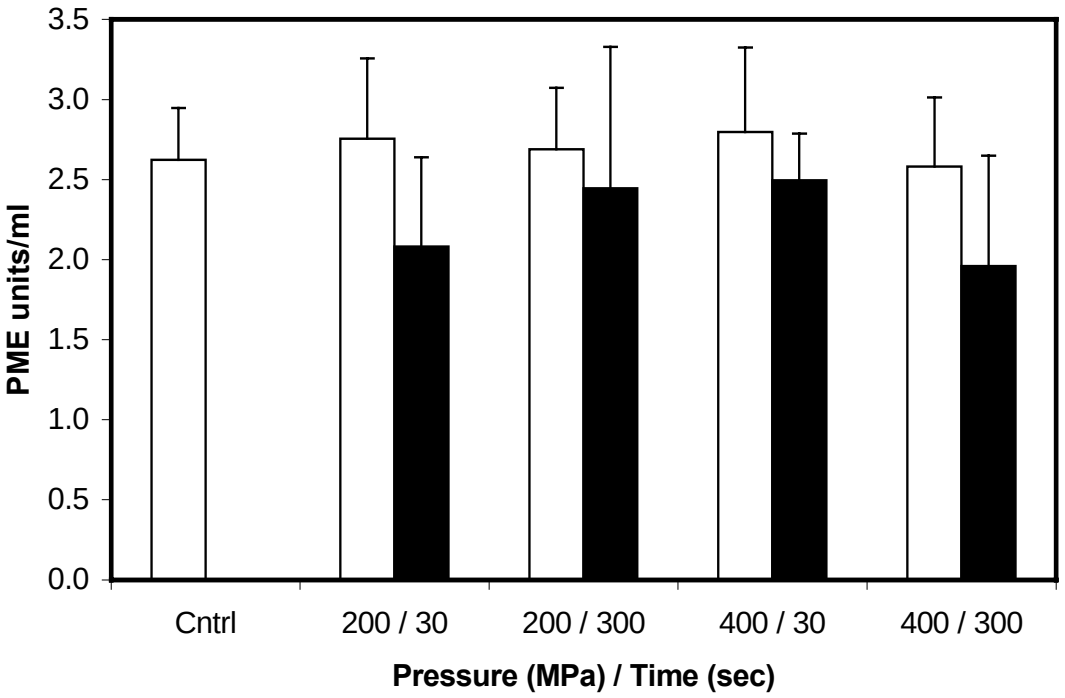


Figure 6.2. Pressure and time effects on PME activity at 15.7±0.6 °C, (□**)** non-carbonated juice, (■) juice carbonated to 9.6±1.4 psig. Error bars represent ± one standard deviation, n=6.

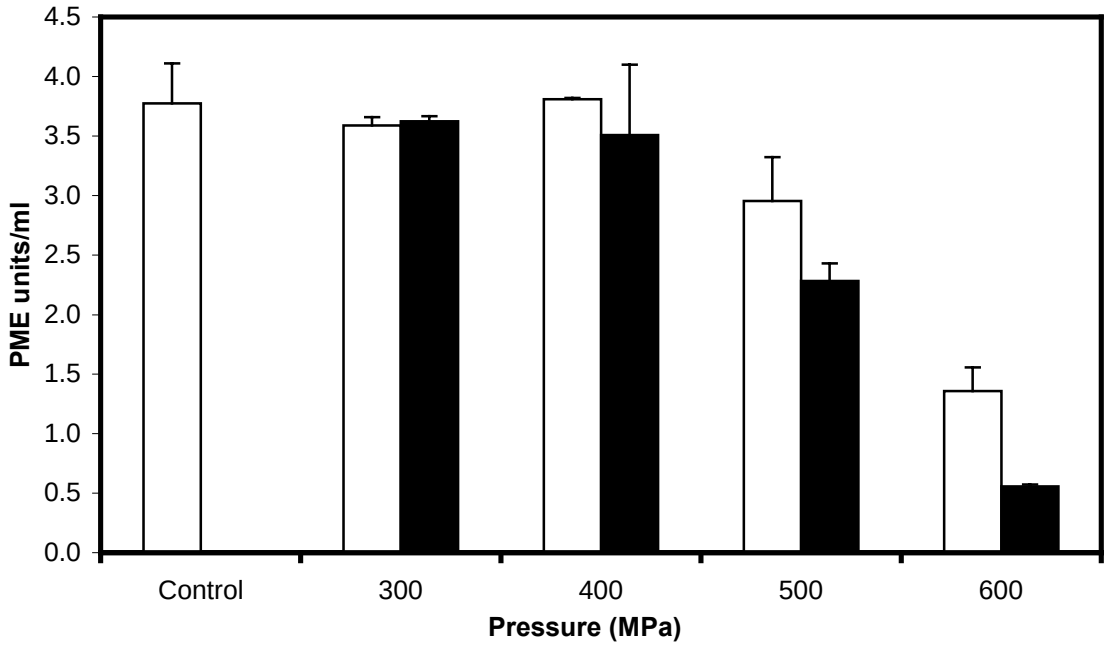


Figure 6.3. Pressure effects on PME activity at 20.0±1.8 °C and 5 min; (□) non-carbonated juice, (■) juice carbonated to 10.1±0.5 psig. Error bars represent ± one standard deviation, n=2.

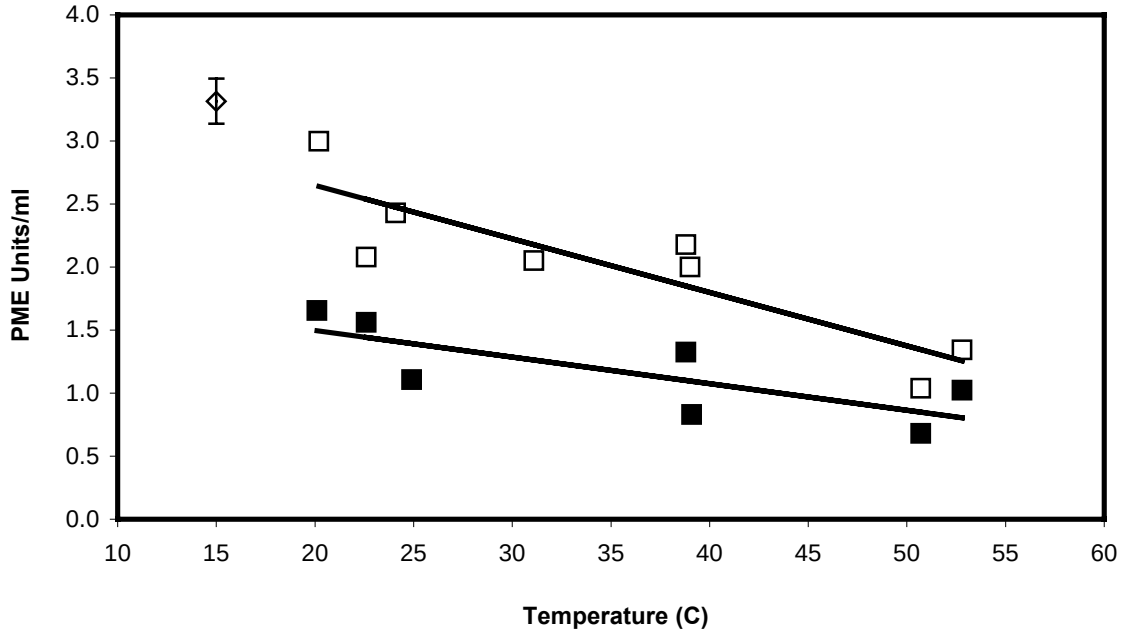


Figure 6.4. Temperature effects on PME activity at 500 MPa, and 5 min; (□) non-carbonated juice, (■) juice carbonated to 11.2±1.0 psig, (◇) control juice. Error bars represent ± one standard deviation, n=2.

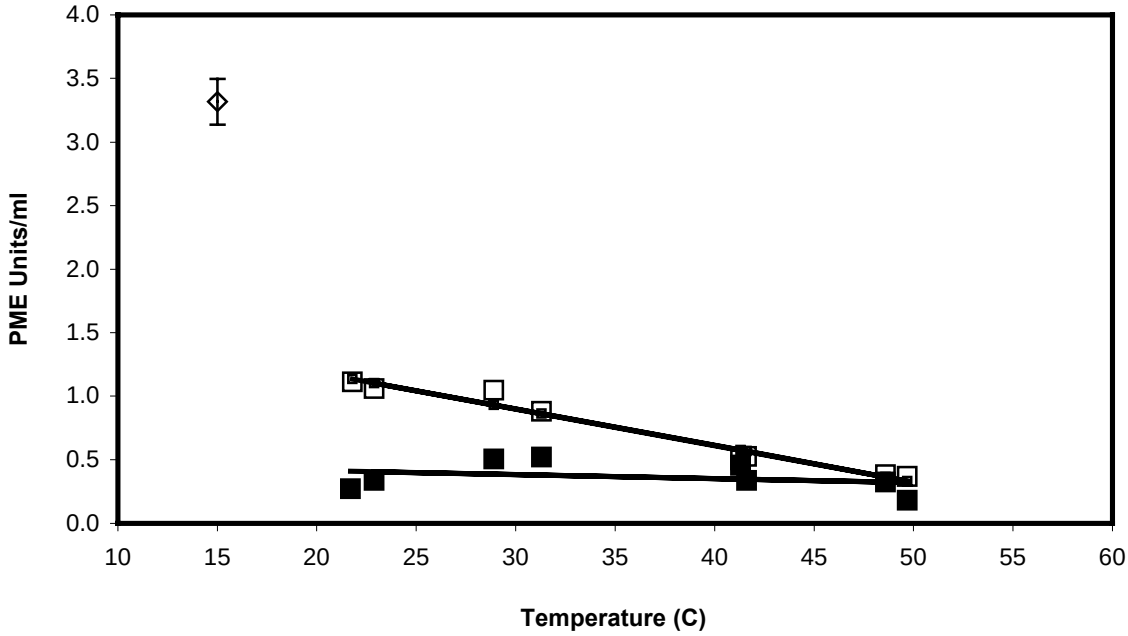


Figure 6.5. Temperature effects on PME activity at 600 MPa, and 5 min; (□**)** non-carbonated juice, (■) juice carbonated to 11.7±1.3 psig, (◇) control juice. Error bars represent ± one standard deviation, n=2.

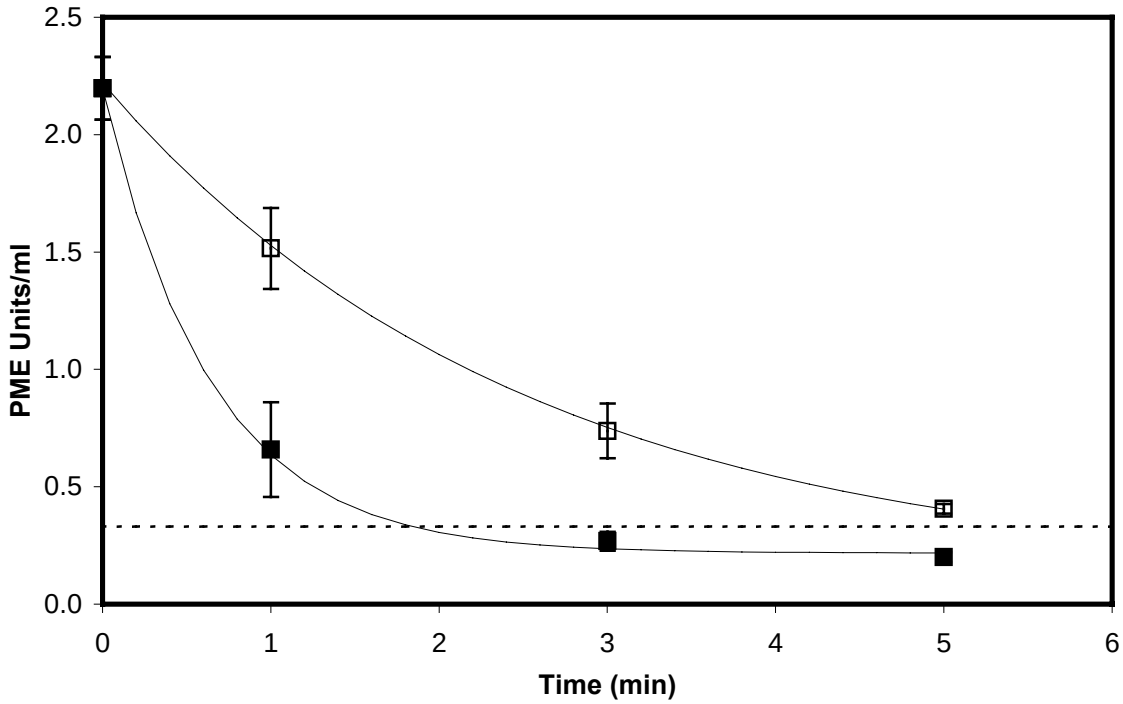


Figure 6.6. Effect of dwell time on PME activity at 600 MPa, and 25 °C; (□**)** non-carbonated juice, (■) juice carbonated to 12.3±0.9 psig. The dash line represents 15% residual PME activity, or 85% PME inactivation. Error bars represent ± one standard deviation, n=4.

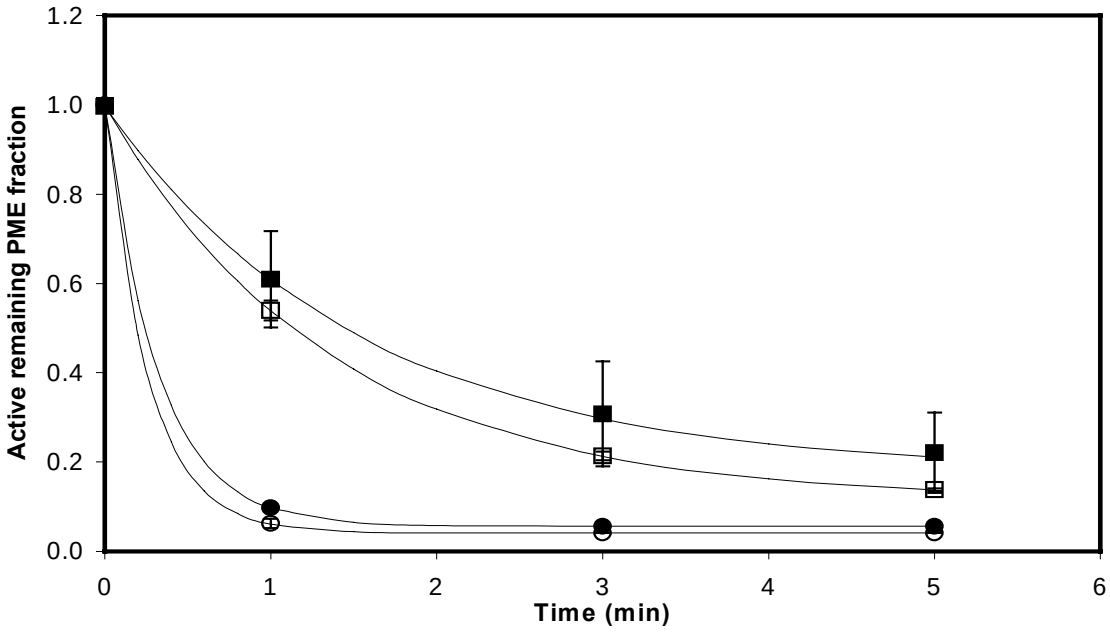


Figure 6.7. Effects of reduced juice pH on PME inactivation at 600 MPa, and 24.7±0.8 °C. Control juice, (■), high pressure treated non-carbonated juice (□), lowered (-0.5) pH 3.27 juice, (●), lowered (-1.0) pH 2.77 juice, (○). Error bars represent ± one standard deviation, n=2 - 4.

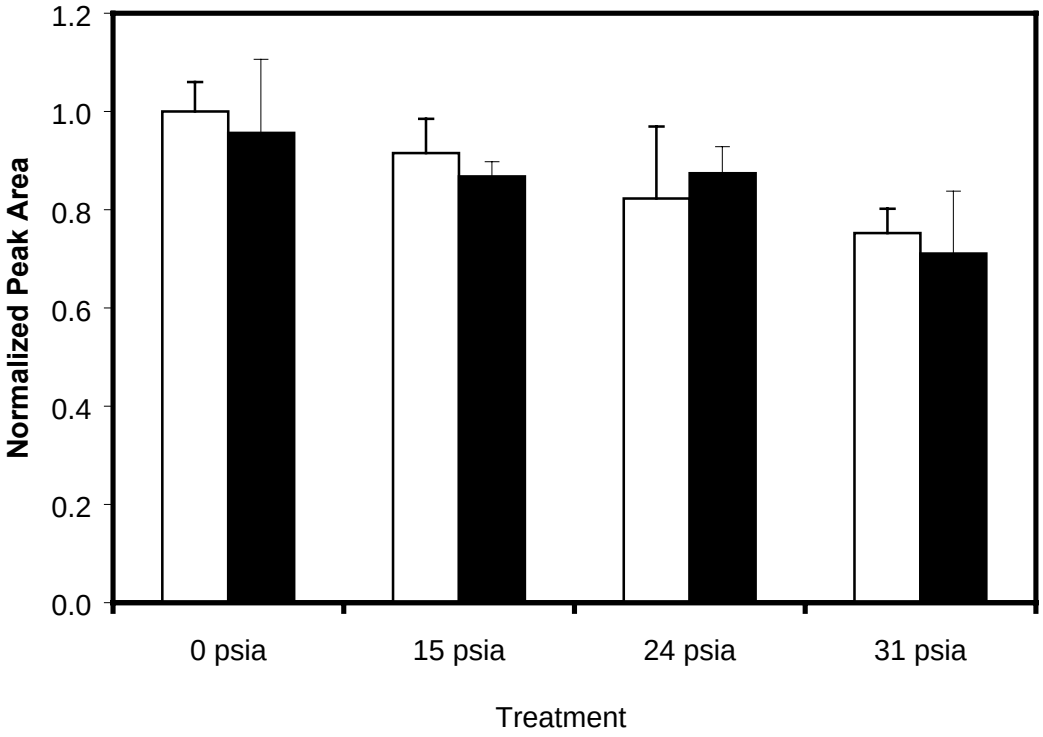


Figure 6.8. Effect of HPP and carbon dioxide on the total volatiles of treated orange juice. Non-pressure treated juice (□). High pressure treated juice (■). Error bars represent ± one standard deviation, n=6.

CHAPTER 7

EFFECT OF THERMAL PROCESSING AND CARBON DIOXIDE-ASSISTED HIGH-PRESSURE PROCESSING ON PECTINMETHYLESTERASE AND CHEMICAL CHANGES IN ORANGE JUICE

7.1. Abstract

The effects of high-pressure processing (HPP), CO_2-assisted high-pressure processing (HPP+CO_2), and thermal processing on the chemical and physical properties of single-strength Valencia orange juice were evaluated over 4 months of storage at 4 and 30 ºC. The HPP+CO_2 juice had the greatest cloud stability and highest ascorbic acid retention. Volatile compound losses were lowest in the HPP juice and lower in the HPP+CO_2 juice compared to the thermally processed juice ($p<0.05$). HPP+CO_2 produced a cloud-stable orange juice with more ascorbic acid and flavor volatiles than thermally processed juice ($p<0.05$).

7.2. Introduction

Orange juice is the most predominant fruit juice in the U.S. market today, with over 94% of the Florida orange juice crop used for orange juice production (Baker and Cameron 1999; Moshonas and Shaw 2000). Commercial orange juice is thermally

processed to inactivate pectinmethylesterase (PME) and spoilage organisms. Active PME will cause clarification of orange juice, which is considered a product quality defect by industrial standards (Braddock 1999). Thermal processing can be detrimental to the organoleptic and nutritional qualities of the juice (Klavons and others 1994; Braddock 1999).

High-pressure processing (HPP) can inactivate the heat-sensitive PME isozyme, and kill spoilage microorganisms, such as lactic acid bacteria, yeasts, and molds (Ogawa and others 1990; Basak and Ramaswamy 1998; Goodner and others 1998; Nienaber and Shellhammer 2001). Elevated pressures in combination with high temperatures have been shown to be ineffective at completely inactivating PME activity in orange juice, leaving behind at least 10% residual PME activity after processing (Ogawa and others 1992; Basak and Ramaswamy 1998; Goodner and others 1998; Van den Broeck and others 1999; Nienaber and Shellhammer 2001). There is compelling evidence suggesting that the heat-stable PME isozyme is also pressure-stable. Nienaber and Shellhammer (2001) have shown that residual PME activity did not possess the same behavior on the juice cloud after processing. The activity of residual PME in orange juice after HPP needs further investigation and elucidation.

Balaban and others (1991) demonstrated that the use of supercritical CO_2 can inactivate PME and render a stable cloud in orange juice without the use of extremely high pressures. Studies have found that adding carbon dioxide gas can lower juice pH during the pressurization process (Balaban and others 1991; Meyssami and others 1992). Previous research conducted in this lab showed that the addition of CO_2 to orange juice prior to HPP resulted in decreased volatiles after processing relative to the HPP alone

(Boff and others 2001). Low-molecular-weight compounds were shown to be lost more readily than higher-molecular-weight compounds as the amount of added CO_2 increased. A comparison of volatile losses due to CO_2-assisted high-pressure processing (HPP+ CO_2) versus thermal processing has yet to be conducted. While CO_2-assisted HPP of orange juice has been shown to have positive effects on PME inactivation and microbial stability (Truong and Shellhammer 2001; Corwin and Shellhammer 2002), its effect on physical and chemical properties during storage have yet to be assessed.

The flavor of orange juice is predominantly derived from its volatile compounds, which are heat-labile (Steffan and Pawliszyn 1996). Numerous studies have identified and assessed the contribution of various volatile compounds to the flavor and aroma of orange juice (Shaw and others 1993; Moshonas and Shaw 2000; Farnworth and others 2001). Qualitative and quantitative analysis of volatiles may be used to assess the flavor quality of orange juice (Moshonas and Shaw 2000). During storage, volatile and ascorbic acid losses will occur in fresh and processed juice; such losses can be dependent on storage temperature, duration of storage and packaging (Ahrne and others 1996; Lee and Coates 1999; Moshonas and Shaw 2000; Yeom and others 2000).

The objective of this study was to compare the effects of CO_2-assisted HPP to HPP and thermally processed Valencia orange juice over a 4-month-storage period at 4 and 30 ºC. Effects of processing and storage were evaluated by measuring juice volatiles, ascorbic acid content, cloud stability, color, titratable acidity, and soluble solids.

7.3. Materials and Methods

Juice extraction and preparation

Orange juice was prepared using the Fresh 'N Squeeze Multi-Fruit juicer (FMC, Lakeland, FL) on fresh Valencia oranges that had been shipped to Columbus, Ohio, from Florida at 4 °C. The fresh juice was filtered with a stainless steel wire-mesh strainer (2 mm) to remove excess pulp and packed in 1-L nylon/EVOH pouches (Winpak Ltd., Winnipeg, Manitoba, Canada). The pouches were quickly frozen and stored at -40 °C until ready for use. Removing the excess pulp was necessary for controlling gas concentration and bottle headspace during the filling and bottling of the CO_2-treated juice, and to ensure an even distribution of enzyme concentration in all samples.

CO_2 gas addition

The thawed orange juice was transferred to a 3-gallon stainless steel mini-keg (N.S.D.A-V.S–0, Spartanburg Steel Products, Inc., Spartanburg, SC). Carbon dioxide was added to the vessel to a pressure of 12 psig (Figure 7.1). The vessel remained in an ice water bath for 30 min with periodic agitation. The gassed juice was filled under pressure into 250-mL polyethylene (PET) bottles (Novapak, Hazleton, PA) using the Zahm Model 9000-R filler (Zahm & Nagel, Inc., Buffalo, NY).

High-pressure processing

Pressure treatment of the juice in the 250-mL polyethylene bottle was performed using an ABB Quintus Food Processor QFP-6 Cold Isostatic Press (ABB Autoclave Systems, Columbus, OH). The pressure fluid was a 1:1 mixture of distilled water and Houghto-Safe 620-TY, containing glycol derivatives (Houghton International, Valley Forge, PA). The bottled juice was pre-chilled in the glycol mixture to 4 °C. The high-pressure process was initiated with the product at approximately 7 °C to compensate for adiabatic heating, which was roughly 3 °C per 100 MPa pressure. The HPP juice was processed at 600 MPa and 25 °C for 346 s, while the HPP+ CO_2 juice was processed at 600 MPa and 25 °C for 130 s. The HPP and HPP+ CO_2 juices were processed for 130 or 346 s in order to achieve equivalent residual PME activity. Addition of CO_2 increases the efficacy of the HPP treatment and shortens the pressure treatment time (Truong and Shellhammer 2001). The pressure, product temperature, and external water-jacket temperature were monitored and recorded in 3-s intervals using a 21X Micrologger (Campbell Scientific Inc., Logan, Utah) connected to a computer with PC208W datalogger support software (Campbell Scientific Inc., Logan, UT).

Thermal processing

Preliminary experiments to determine heating from 0 °C to 91 °C and cooling times from 90 °C to 0 °C were performed using nylon/EOVH pouches filled with 500 mL fresh juice. A copper/constantan duplex ANSI type T thermocouple was introduced into the

center of the pouch, and the pouch was heat-sealed without headspace. Pouches were immersed in 150 L water (91 °C) in a steam-jacketed kettle. After heating, the pouches were rapidly cooled in an ice-water bath. Temperature readings were recorded manually every 15 s during heating, holding, and cooling times.

The heating come-up time of the juice inside the nylon/EVOH pouches from 0 °C to 91 °C was 80 s as determined by heat transfer calculations and verified with the heating trials. Eagerman and Rouse (1976) reported that complete inactivation of PME in orange juice occurs at 90 °C for 1 min hold time. However, a 1-min hold time at 90 °C is excessive by industrial standards, and results in significant quality loss of the juice. A 30-s hold time at 91 °C was selected as it was sufficient to render <0.5% residual PME activity, while incurring minimal damage to the quality of the juice. Total heating time of the thermally processed juice for this study was 110 s.

Cloud loss

Cloud loss was determined by an industrial quality control method developed by Redd and others (1986). A 50-mL sample of juice was centrifuged at 360 x g for 10 min using a tabletop centrifuge (Dynac II, Becton Dickinson, Sparks, MD). The percent transmission (% T) of the supernatant was measured by spectrophotometer at 650 nm (Shimadzu, Inc., Wood Dale, IL).

Pectinmethylesterase assay

PME activity of degassed juice was determined titrimetrically at pH 7.7 in a 30 °C agitated water bath using the method of Rouse and Atkins (1955). An initial addition of 2.0 N NaOH was used to raise the pH of the juice to pH 7.7. Addition of 0.05 NaOH from an automatic burette was used to maintain the pH at appropriate levels using a Computer-Aided Titrimeter titration system (Fisher Scientific, Pittsburgh, PA). The reaction mixture consisted of 10 mL juice and 40 mL of 1% (10 g/L) pectin (P 9135; Sigma, St. Louis, MO) solution containing 0.1 M NaCl (Fisher Scientific). Each sample was analyzed in duplicate, analysis time varied from 5 to 20 min depending on the level of PME activity. PME activity was calculated by the following formula:

$$\text{PME units/mL} = \frac{(\text{mL NaOH}) \cdot (0.05 \text{ N NaOH}) \cdot (1000)}{(10 \text{ mL sample}) \cdot (\text{time})}$$

Color, total acidity, and soluble solids

L (lightness), a (redness), and b (yellowness) values were measured with a HunterLab Ultrascan spectrophotometer (Hunter Associates Laboratory, Reston, VA). Samples were filled into glass cuvets with a 2-cm path length. The following settings were used: reflectance mode, illuminant A, 10 ° observer.

Each total color differences (DE) value was calculated as:

$$DE = ((L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2)^{0.5}$$

Titratable acidity (as percent citric acid) was measured using the method of Redd and others (1986). A 10-mL sample of juice was mixed with 90 mL distilled water. The

test was conducted with a Computer-Aided Titrimeter (CAT) titration system (Fisher Scientific) by addition of 0.31 N NaOH from an automatic burette to an endpoint of pH 8.2. The soluble solids (°Brix) was measured using the ABBE refractometer (Warner-Lambert, Inc., Parsippany, NJ).

Ascorbic acid analysis

Ascorbic acid was measured by the HPLC procedure of Yeom and others (2000). A sample of the treated orange juice was placed in a micro centrifuge tube and centrifuged for 10 min at 10,000 rpm. The supernatant was filtered through a 0.45-μm disk filter. Ascorbic acid in the filtered supernatant was measured using a Hewlett-Packard 1050 liquid chromatograph equipped with a C-18 column (5 μm particle size, 4.6 mm diameter, 250 mm length) with a C-18 guard column, and a UV detector set at 254 nm. The mobile phase was a 10:90 (v/v) mixture of methanol and water. The water was acidified to pH 2.8 using phosphoric acid (0.01%, v/v). The mobile phase was filtered with a 0.45 μm membrane filter and degassed using helium. Peak area was calculated using a Hewlett-Packard HP 3396A integrator (Wilmington, DE). Duplicate measurements were made for each sample.

Volatile analysis

Volatiles were collected and analyzed by headspace solid-phase microextraction gas chromatography (SPME-GC) using a procedure modified from Jia and others (1998). Comparison of retention times of standards (Aldrich Chemical Co., Milwaukee, WI), as

well as mass spectroscopy, was used to identify chemicals. A 2-mL aliquot of the treated orange juice was placed in a 10-mL glass vial and sealed with a Teflon-coated rubber septum and an aluminum crimp cap (Supelco, Inc., Bellefonte, PA). Prior to use, the SPME fiber was conditioned for 60 min at 250 °C according to the manufacturer's specifications. Volatile compounds were extracted via SPME using a 100-μm polydimethylsiloxane (PDMS)-coated fiber (Supelco) by manual insertion into the vial headspace for 30 min at 40 °C. Extracted volatiles were desorbed in the GC injection port for 5 min at 250 °C. The injection port was fitted with a 0.75-mm internal diameter splitless glass liner (Supelco). Volatile compounds were separated and detected using an HP-5 column (crosslinked 5% PH ME Siloxane) 30m x 0.32mm x 0.25μm film thickness (Supelco) in a flame ionization detector -quipped Hewlett-Packard 5890 GC (Wilmington, DE) using nitrogen carrier gas. The GC oven was held at 60 °C for 2 min, increased at 6 °C/min to 150 °C, increased at 10 °C/min to 220 °C and held for 5 min at 220 °C. Peak area was calculated using a Hewlett-Packard HP 3396A integrator (Wilmington, DE). Duplicate measurements were made for each sample.

Microbial analysis

The presence of microorganisms in the juice samples was determined through direct plating on selective and nonselective microbial media. Serial dilutions in sterile 0.1% peptone water were performed and survival of lactic acid bacteria was determined by spread-plating 0.1 mL of the samples on MRS agar plates. Total microbial population was estimated by spread-plating 0.1 mL samples on total plate count agar. Two dilutions

of each sample were plated in duplicate and were counted after 48 hr of aerobic incubation at 37 °C. All media for enumeration of microbial samples were purchased from Difco.

Storage study design

After processing the juice by either thermal, HPP, or HPP+ CO_2, the juices were aseptically transferred to 60-mL Qorpak amber glass vials and sealed with Teflon TFE fluorocarbon resin liner screw caps (Fisher Scientific) in a laminar flow hood to minimize contamination. The juice repacking process was performed under ambient conditions thus resulting in some carbon dioxide degassing. Based on the repacking temperature and pressure (10 °C, 101.325 kPa), the new CO_2 concentration in the juice was estimated to be 0.09 molar percent (0.22 % w/w). The bottles were stored in incubators at 4 ± 0.36 °C and at 30 ± 0.19 °C. Ascorbic acid, volatiles, color, and $\%T_{650}$ was measured at 0, 2, 7, 14, 30, 60, 90, and 120 days. Ascorbic acid, volatiles, color, $\%T_{650}$, soluble solids, pH, and titratable acidity of the fresh, unprocessed juice was measured initially, but no samples of the unprocessed juice were held for storage due to rapid spoilage.

Statistical analysis

Averages of duplicate measurements from 3 independent sets of processed juice were used for statistical analyses. SAS (SAS system for Windows, release 7, SAS Institute, Cary, NC) was used for analysis of variance (PROC GLM), correlation (PROC CORR)

and regression (PROC REG) analyses. Means of significant factors were compared using Tukey's studentized range ($\alpha = 0.05$).

7.4. Results and Discussion

Unprocessed control juice

The unprocessed juice was measured initially to serve as a control point for flavor and quality changes of the thermally processed, HPP, and HPP+ CO_2 juices. The chemical and physical properties (mean ± one standard deviation) of the control juice were: pH 3.88±0.02, 12.31±0.04 °Brix, 0.33±0.05 g citric acid/ 100 mL juice, 7.26±2.37% T @ 650 nm, and L,a,b values (47.22±0.93, 10.57±0.93, 13.05±0.49). The PME concentration in the control juice (3.2 PME units/mL) was similar to other PME studies (Goodner and others 1998; Van den Broeck and others 1999; Nienaber and Shellhammer 2001). After processing, the thermally processed juice had 0.8±0.32% residual PME activity; the HPP juice had 20.2±2.7%; and the HPP+CO_2 juice had 27.4±1.47% (Table 7.1). The pH of all juices was unchanged by the specific processing technique.

Juice cloud stability

Commercial thermal processing is an effective method to inactivate heat-stable and heat-labile PME in orange juice. Heating the juice at 90 °C for 1 min will inactivate 99% of the heat-stable PME isozyme. The heat-stable PME is primarily responsible for clarification of the orange juice cloud (Eagerman and Rouse 1976; Versteeg and others 1980). The heat-stable PME can make up 5% of the total PME concentration in Valencia

orange juice, and any remaining active TS-PME fraction will clarify the juice during storage (5 °C) in less than 4 weeks (Versteeg and others 1980; Wicker and Temelli 1988; Cameron and others 1997). Since the thermally processed juice in this study had only 0.8% residual PME activity, it was expected that the juice cloud would remain stable for the 4-month duration of the shelf life study. The % T values (2.5 to 3%) of the thermally-processed juice cloud were lower (more turbid) than the HPP juice (4 to 7%), and higher (less turbid) than the HPP+CO_2 juice (1 to 1.5%) at 4 °C storage temperatures ($p<0.05$) (Figure 7.2).

Even 5% residual PME activity can destabilize the juice cloud. However, Goodner and others (1998) and Nienaber and Shellhammer (2001) demonstrated that HPP juice cloud was stabilized for >55 days at 4 °C with ≥10% residual PME activity during storage. While in the present study high levels of residual PME activity were present in the HPP juice (20.2±2.7%) and HPP+ CO_2 juice (27.4±1.47%), both juices remained physically stable at 4 and 30 °C during the 4-month storage period. If a thermally-processed juice had >20% residual PME activity, complete cloud lost would occur within 4 weeks (Versteeg and others 1980; Wicker and Temelli 1988; Cameron and others 1997). During storage, the HPP juice cloud was less turbid than the thermally processed juice at 4 °C storage temperature suggesting that its cloud was not as stable as the thermally- processed juice. However, the transmission levels were still well below the 36% T cutoff used by the industry to indicate a loss of cloud.

The % T of the thermally-processed, HPP and HPP+CO_2 juices were 2.94±0.45% T, 5.29±1.01% T, and 0.95±0.08% T, respectively, at 4 °C. The % T of the thermally

processed, HPP and HPP+CO_2 juices were 2.55±0.69% T, 2.80±0.93% T, and 0.69±0.17% T, respectively, at 30 °C. As a measure of juice quality, Redd and others (1986) categorized juices with ≥36% T reading at 650 nm in the supernatant after centrifugation as "definite" cloud loss; 25% - 35% as "slight" cloud loss; and 0% - 24% as "no" cloud loss. Using these values, all juice treatments had a stable cloud. In other words, "no" cloud loss had occurred during the storage period at 4 or 30 °C.

Effect of processing method on off color development

Browning development in orange juice has been correlated with ascorbic acid loss/degradation (Kaanane and others 1988; Kaanane 1992; Nienaber and Shellhammer 2001). Donsì and others (1996) and Nienaber and Shellhammer (2001) reported that HPP does not cause significant changes in juice color stored at temperatures < 8 °C. Juice stored at 4 °C had higher (L) values than the samples stored at 30 °C, slightly lower (a) values at 4 °C than 30 °C, and higher (b) values at 4 °C than 30 °C (Figure 7.3). Off color development in the juice due to processing method was not statistically significant ($p>0.05$), however, storage temperature was a significant factor ($p<0.05$) (Figure 7.4). Browning in the samples stored at 30 °C was likely due to nonenzymatic browning reaction, which is accelerated at higher temperatures. A 30 °C storage temperature produced more off color and lower % T values than the juice stored at 4 °C, independent of processing method.

Ascorbic acid

Storage time and temperature had a significant effect ($p<0.05$) on the loss of ascorbic acid (Table 7.2). Average ascorbic acid content of all juices stored at 4 °C was 19% higher than the samples stored at 30 °C. Processing method did not significantly affect ascorbic acid loss at either 4 °C or 30 °C storage through 14 days ($p>0.05$). However, the HPP+CO_2 sample had less ascorbic acid loss than the HPP juice when stored at 4 °C after 28 days ($p<0.05$, Figure 7.5). Ascorbic acid degradation has been characterized by simultaneous aerobic and anaerobic reactions, with the aerobic degradation being the fastest (Ahrne and others 1996). The addition of CO_2 to orange juice may be beneficial to ascorbic acid retention due to the displacement of dissolved oxygen from the liquid matrix. The use of HPP+ CO_2 can be beneficial to ascorbic acid retention during storage, especially during the later stages of storage at 4 °C.

Analysis of headspace volatiles

The loss of many volatile compounds was lower in the HPP and HPP+ CO_2 juices compared to the thermally-processed juice (Table 7.2). Low-molecular-weight compounds such as α-pinene (Figure 7.6), β-myrcene (Figure 7.7), and limonene were significantly higher in the HPP and HPP+ CO_2 juices than in the thermally-processed juice ($p<0.05$). HPP without added CO_2 generally had the lowest loss of volatile compounds. α-Pinene was significantly higher ($p<0.05$) in the HPP and HPP+ CO_2 juices compared to the thermally-processed juice through 14 days (Figure 7.6). α-Pinene was unaffected by HPP and HPP+ CO_2 processing initially, however, the thermally-

processed juice measured 19% lower in α-pinene relative to the unprocessed juice. α-Pinene was unaffected by storage temperature (Table 7.2). β-Myrcene was significantly higher ($p<0.05$) in the HPP and HPP+CO_2 juices through 28 days compared to the thermally-processed juice (Figure 7.7). Linalool losses were significantly lower ($p<0.05$) in the HPP juice compared to the thermally processed and HPP+CO_2 juices (Figure 7.8). At 56 days and beyond, amounts of linalool were statistically similar for all processing conditions ($p>0.05$).

Limonene, which constitutes 80 to 90% of the total volatiles in orange juice, was significantly affected by thermally processing. Compared to the control juice, initial limonene losses in the thermally processed juice, HPP juice and HPP+ CO_2 juice were 39.8%, 12.8% and 16.9%, respectively. Although limonene is by far the major volatile in orange juice, it is not the most important contributor to flavor quality (Jia and others 1998). It can chemically react to form degradation products like α-terpineol, which is recognized as an off-flavor contributing a stale, musty aroma (Moshonas and Shaw 1989). Changes in α-terpineol were independent of the processing procedure at 4 and 30 °C but were highly dependent on the storage temperature (Table 7.2).

Decanal (Figure 7.9), caryophyllene, and valencene were higher in the thermally processed juice than in the HPP juices ($p<0.05$). These comparatively high-molecular-weight compounds may be less sensitive to the thermal processing than the lower-molecular-weight compounds or more sensitive to high-pressure processing. Previous research showed that volatile compounds were more readily lost with increasing levels of CO_2, and that HPP had no significant effect on volatile losses (Boff and others 2001).

Furthermore, the lower-molecular-weight compounds tended to be more easily lost than the higher-molecular-weight compounds with the increase of CO_2. Therefore, the results cannot be explained by sensitivity to HPP.

Microbial enumeration

At the conclusion of the 4-month study, juices from all treatment and storage conditions were sampled for the presence of microbial growth. Little to no microbial growth was detected across all treatment levels (Table 7.3). Results are merely estimates of microbial populations as positive plates yielded only 1 – 3 countable colonies. Changes in chemical and physical properties described earlier were thus not attributable to the growth of spoilage microorganisms.

7.5. Conclusions

Although the HPP and HPP+ CO_2 juices had residual PME activities greater than 20%, both processes produced an orange juice with comparable cloud stability to thermally-processed juice during storage at 4 and 30 ºC for 4 months. Browning of the juice was independent of processing method and dependent on storage temperature. Carbon dioxide-assisted high-pressure processing is beneficial to the ascorbic acid retention compared to conventional thermally-processed juice during storage at 4 ºC, especially beyond 4 weeks. The positive effect of carbon dioxide may be due to the displacement of oxygen from the juice. Carbon dioxide-assisted high-pressure processing had variable effects on orange juice volatiles. Most of the identified low-molecular-weight

compounds had better retention in the HPP and HPP+ CO_2 juices compared to the thermally-processed juice. Losses of ascorbic acid and flavor volatiles can be reduced by carbon dioxide-assisted high-pressure processing compared to conventional thermal processing.

Table 7.1. Percent PME inactivation of various processing treatments. Error bars represent ± 1 standard deviation, n=3.

	Temperature	Pressure	Time	PME
Treatment	**T (°C)**	**p (MPa)**	**t (sec)**	**Reduction**
Thermal	91*	0.1	30**	99.2±0.32%
HPP	25	600	346	79.8±2.70%
HPP + CO_2	25	600	130	72.6±1.47%

* Heat come-up time (0 to 91 °C): 80 s
* *Hold time at temperture

Table 7.2. Significant effects ($p<0.05$) of processing, storage temperature and storage time on ascorbic acid and headspace volatiles.

	Processing condition	Temperature	Storage time
Ascorbic acid	HPP+CO_2 > HPP = Thermal	4 > 30	Decreases over time
Ethyl butyrate	NS	4 > 30	NS
α-Pinene	HPP = HPP+CO_2 > Thermal	NS	Decreases over time
b-Myrcene	HPP = HPP+CO_2 > Thermal	NS	Decreases over time
Limonene	HPP = HPP+CO_2 > Thermal	NS	Decreases over time
Linalool	HPP > HPP+CO_2 = Thermal	4 > 30	Decreases over time
α-Terpineol	NS	30 > 4	Increases over time
Decanal	Thermal > HPP = HPP+CO_2	4 > 30	Decreases over time
Caryophyllene	Thermal > HPP > HPP+CO_2	NS	NS
Valencene	Thermal > HPP > HPP+CO_2	NS	NS

NS indicates no significance ($p>0.05$)

Table 7.3. Microbial enumeration estimates of juice samples (colony forming units/mL).

	Storage temperature			
	4 ºC		30 ºC	
Treatment	TPC	LAB	TPC	LAB
Thermal	5	ND	ND	ND
HPP	ND	ND	ND	ND
HPP + CO_2	15	5	25	ND

TPC = Total plate count; LAB = lactic acid bacteria, ND = nondetectable

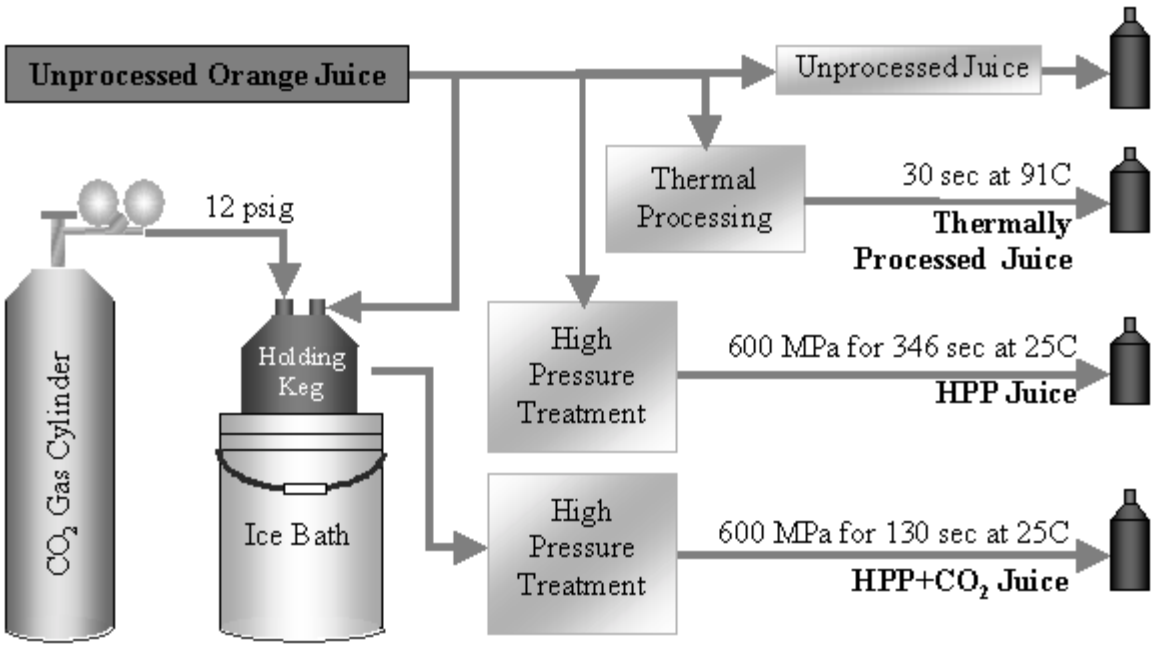


Figure 7.1. Preparation of thermally-processed, high-pressure processed and carbon dioxide-assisted high-pressure processed juices.

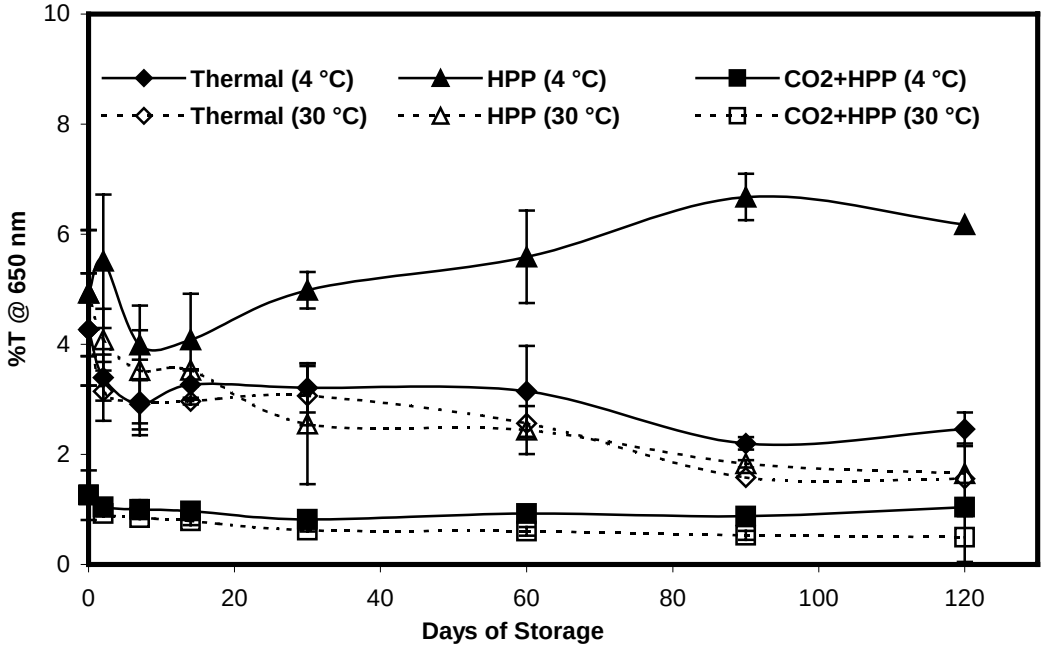


Figure 7.2. Percent transmission of the various treated juices @ 650 nm. Error bars represent ± 1 standard deviation, n=3.

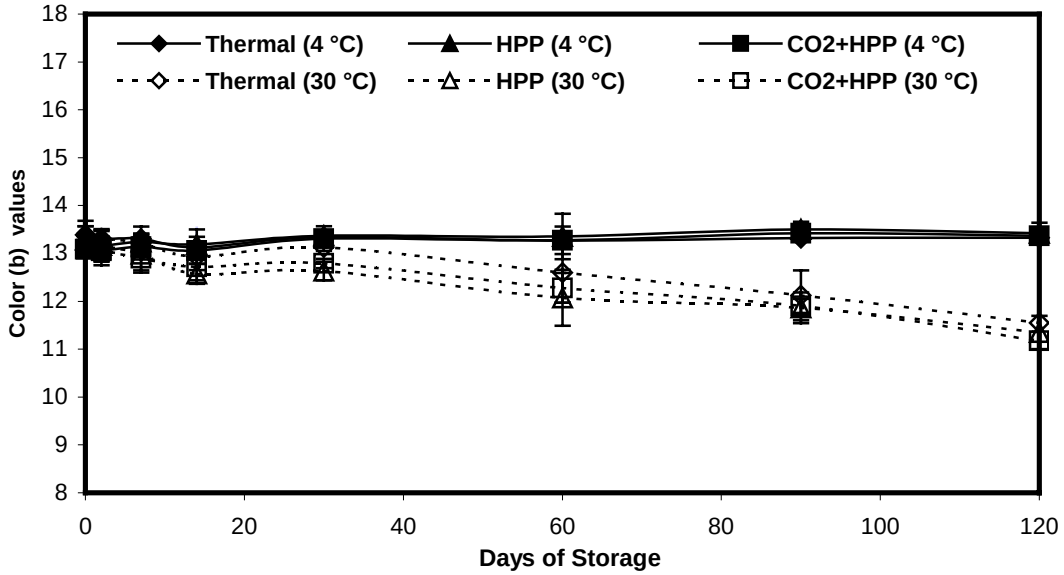


Figure 7.3. Color b (yellowness) value changes of the various treated juices during storage. Error bars represent ± 1 standard deviation, n=3.

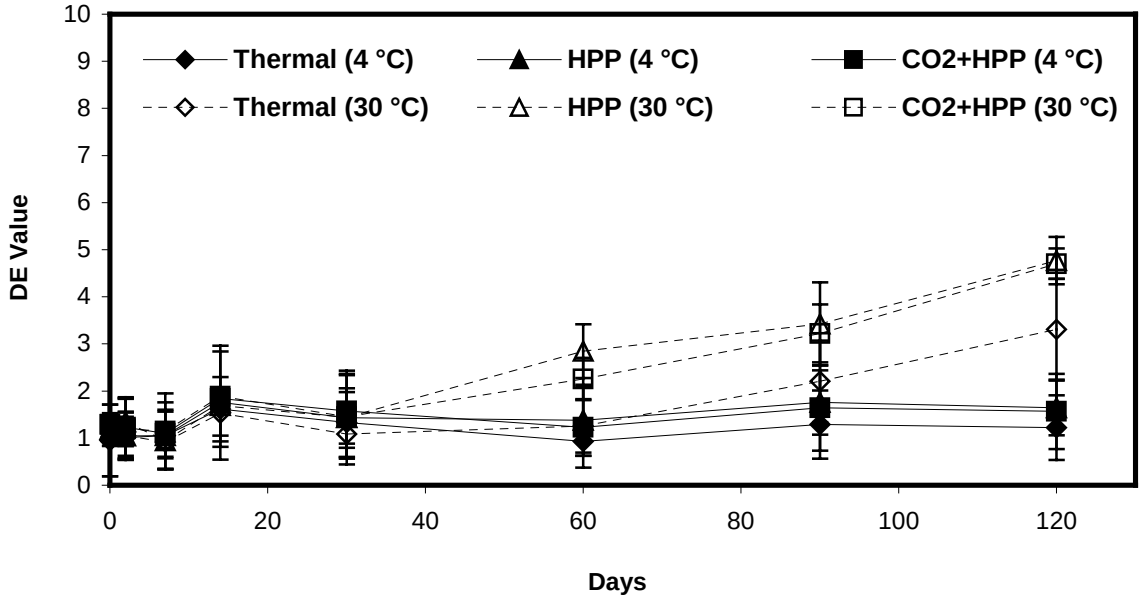


Figure 7.4. Total color difference (DE value) of the various treated juices during storage. Error bars represent ± 1 standard deviation, n=3.

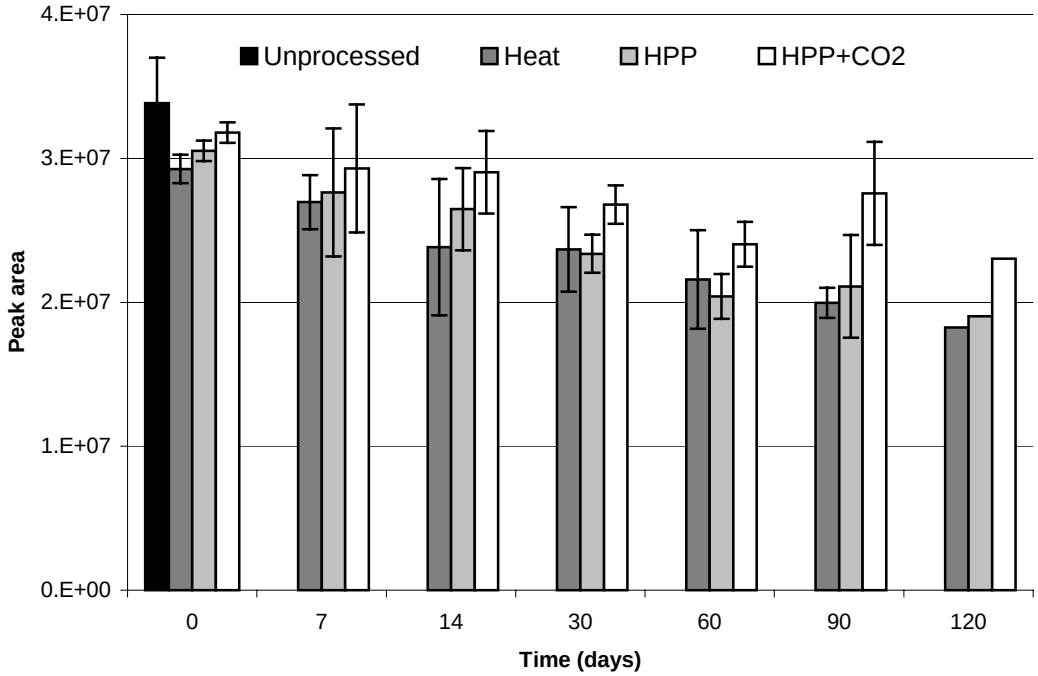


Figure 7.5. Ascorbic acid measurements of samples stored at 4 °C for 120 days. Error bars represent ± 1 standard deviation, n=3.

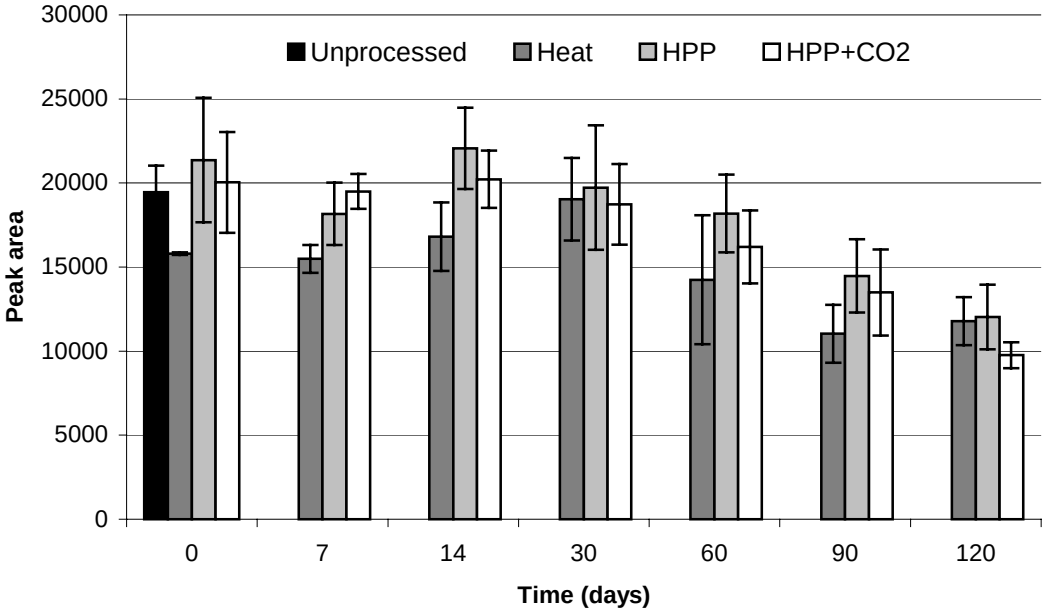


Figure 7.6. α-Pinene in samples stored at 4 and 30 °C for 120 days. Error bars represent ± 1 standard deviation, n=3.

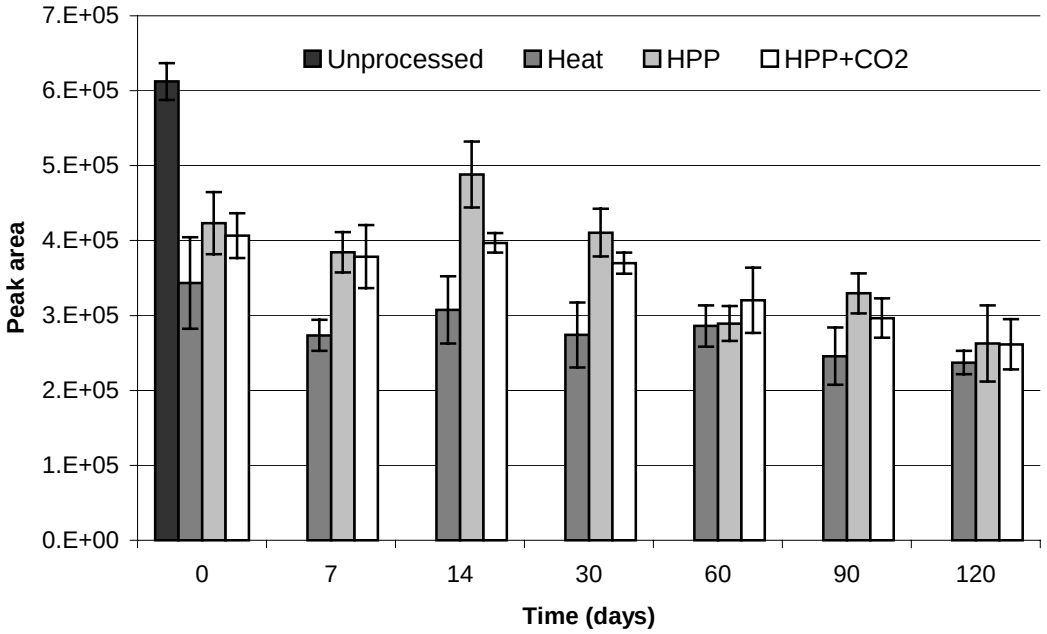


Figure 7.7. β-Myrcene in samples stored at 4 and 30 °C for 120 days. Error bars represent ± 1 standard deviation, n=3.

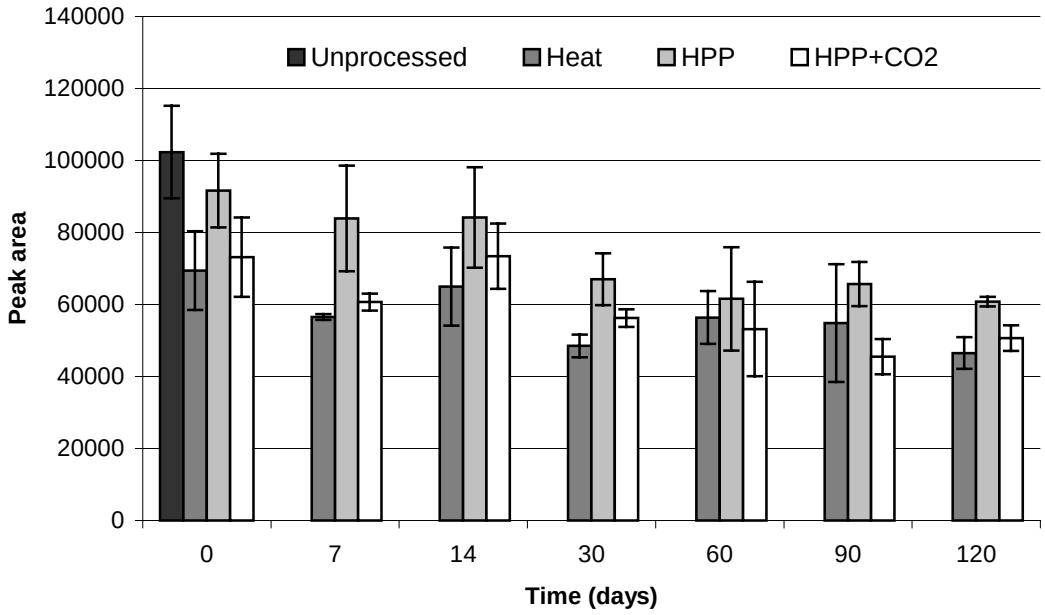


Figure 7.8. Linalool in samples stored at 4 °C for 120 days. Error bars represent ± 1 standard deviation, n=3.

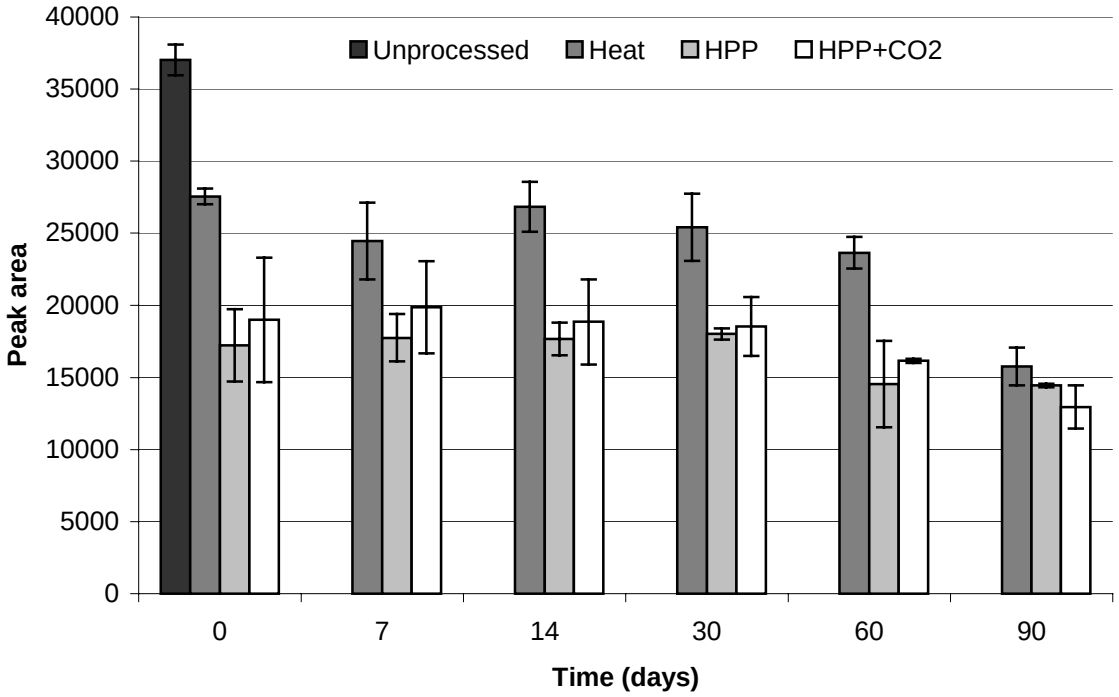


Figure 7.9. Decanal in samples stored at 4 and 30 °C for 120 days. Error bars represent ± 1 standard deviation, n=3.

CHAPTER 8

CONCLUSIONS

8.1. Conclusions: Lipid oxidation

1. The amount of total volatile compounds produced in soybean oil, peanut oil and lard was dependant on storage time, light exposure and chlorophyll concentration. The amount of total volatiles in soybean oil and peanut oil was also dependant on storage temperature.
2. Volatile compounds in soybean oil, peanut oil and lard were qualitatively similar with or without added chlorophyll.
3. Light exposure had a significant effect on the production of almost all volatile compounds in soybean oil, peanut oil and lard.
4. The presence of 5 to 10 ppm chlorophyll present in soybean oil significantly increased the production of approximately half of the volatile compounds in the soybean oil and lard after 1 day of light exposure.
5. Pentane, pentanal, 1-pentenol, 2-pentenal, hexane, 1-hexene, 2-hexanal, heptanal, 2-heptenal, 2-pentyl furan, 2,4,-heptadienal, 2,4-nonadienal, 1-octen-3-ol and 2-octenal were identified as products of singlet oxygen oxidation.

6. The presence of chlorophyll was the most significant factor to the amount of chlorophyll sensitive compounds produced in soybean oil during the first 2 days of light exposure; however, temperature was the most significant factor after 2 days of light exposure.
7. Fatty acid composition was a significant factor to the amounts of total volatiles and hexanal present in soybean oil, peanut oil and lard. Fatty acid composition is also an important factor in photoxidation indicating that autoxidation reactions are an important contributor to the production of volatile compounds imitated by photosensitized reactions.
8. Chlorophyll concentration had no effect on the amount of the triplet oxygen autoxidation product hexanal. The amount of hexanal in soybean oil was dependant on storage temperature, light exposure and the duration of light exposure.
9. 2-Heptenal increased in quantity rapidly at 4 °C in soybean oil and peanut oil containing 5 ppm or 10 ppm chlorophyll that was exposed to light.
10. The rate of increase of 2-heptenal was several times greater than the rate of increase of hexanal in soybean oil, peanut oil and lard containing 5 ppm or 10 ppm chlorophyll, due to the high reactivity of singlet oxygen.

8.2. Conclusions: Carbon dioxide assisted high pressure processed orange juice

1. Pressure processing did not significantly alter the volatile profile of fresh, single strength orange juice, but did result in minor reductions in ascorbic acid.
2. Carbon dioxide-assisted high-pressure processing is beneficial to the ascorbic acid retention compared to conventional thermally-processed juice during storage at 4 ºC,

particularly beyond 4 weeks. The positive effect of carbon dioxide may be due to the displacement of oxygen from the juice.

3. The addition of carbon dioxide to the process significantly affected orange juice volatiles, and this phenomenon is hypothesized to be due to the method of gassing and degassing the juice. Most of the identified low-molecular-weight compounds had better retention in the HPP and HPP+ CO_2 juices compared to the thermally-processed juice.
4. Combining carbon dioxide with high pressure processing of fresh orange juice can significantly increase the rate of PME inactivation beyond that achieved by pressure alone. The implication of this finding is potentially shorter dwell times at reduced pressures. Although the HPP and HPP+ CO_2 juices had residual PME activities greater than 20%, both processes produced an orange juice with comparable cloud stability to thermally-processed juice during storage at 4 and 30 ºC for 4 months.

BIBLIOGRAPHY

Adam W. 1975. Singlet molecular oxygen and its role in organic peroxide chemistry. Chem. Ztg 99:142-155.

Afonso SG, Enriquez de Salamanca R, and del C Batlle AM. 1999. The photodynamic and non-photodynamic actions of porphyrins. Brazilian Journal of Medical and Biological Research 32:255-266.

Ahrne LM, Manso MC, Shah E, Oliveira FAR, Oste RE. 1996. Shelf-life prediction of aseptically packaged orange juice. In: Lee TC Kim HJ, editors. Chemical markers for processed and stored foods. American Chemical Society: Washington, DC.

Allen C and Parks OW. 1975. Evidence for methional in skim milk exposed to sunlight. J Dairy Sci 58:1609-1611.

Allen C and Parks OW. 1979. Photodegradation of riboflavin to lumichrome in milk exposed to sunlight. J Dairy Sci 60:1038-1041.

Andersen LK and Ogilby PR. 2001. Time-resolved detection of singlet oxygen in a transmission microscope. Photochem Photobiol 73:489-492.

Ando T, Yoshikawa T, Tanigawa T, Kohno M, Yoshida N, and Motoharu K. 1997. Quantification of singlet oxygen from hematoporphyrin derivative by electron spin resonance. Life Sciences 61:1953-1959.

Athar M, Mukhtar H, and Bickers DR. 1988. Differential role of reactive oxygen intermediates in photofrin-I-initiated and photofrin-II-initiated photoenhancement of lipid peroxidation in epidermal microsomal membranes. Journal of Investigative Dermatology 90:652-657.

Baker RA, Cameron RG. 1999. Cloud of citrus juices and juice drinks. Food Tech 53(1):64-69.

Balaban MO, Arreola AG, Marshall MM, Peplow A, Wie CI, Cornell J. 1991. Inactivation of pectinesterase in orange juice by supercritical carbon dioxide. J Food Sci 56(3):743-746.

Balance PE. 1961. Production of volatile compounds related to the flavour of foods from the strecker degredation of DL-methionine. J Sci Food Agric 12:532-536.

Basak S, Ramaswamy HS. 1998. Ultra high pressure treatment of orange juice: a kinetic study on inactivation of pectin methyl esterase. Food Res Int 29(7):601-607.

Beutner S, Bloedorn B, Hoffman T, and Martin HD. 2000. Synthetic singlet oxygen quenchers. In: Packer L and Sies H, editors: Methods in Enzymology Volume 319. New York: Academic Press. 226-241.

Bisby RH, Morgan CG, Hamblett I, and Gorman AA. 1999. Quenching of singlet oxygen by trolox C, ascorbate and amino acids: effects of pH and temperature. J Phys Chem 103: 7454-7459.

Boff JB, Shellhammer TH, Min DB. 2001. The impact of CO_2-assisted high-pressure processing on fresh orange juice flavor and ascorbic acid content. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 25.

Braddock RJ. 1999. Single strength orange juices and concentrate. In: Braddock RJ, editor. Handbook of Citrus By-Products and processing Technology. Wiley: New York. P 53-83.

Bradley DG. 1991. Riboflavin photosensitized singlet oxygen oxidation in milk products. Ph.D. Dissertation. The Ohio State University, Columbus.

Bradley DG and Min DB. 1992. Singlet oxygen oxidation of foods. Crit Rev Food Sci Nutr 31:211-236.

Brekke OL. 1980. In: Erickson DR, Pryde EH, Brekke OL, Mounts TL and Falb RA, editors. Handbook of soyoil processing and utilization. Champaign, IL: American Oil Chemists' Society. p 109.

Callison AL. 2001. Singlet oxygen lipid oxidation mechanisms for reversion flavor formation in soybean oil. M.S. Thesis. The Ohio State University, Columbus.

Cameron RG, Baker RA, Grohmann K. 1998. Multiple forms of Pectinmethylesterase from citrus peel and their effects on juice cloud stability. J Food Sci 63:253-256.

Cano MP, Hernandez A, DeAncos B. 1997. High pressure and temperature effects on enzyme inactivation in strawberry and orange products. J Food Sci 62:85-88.

Chan HWS. 1975. Artificial food colours and the photooxidation of unsaturated fatty acid methyl esters: the role of erythrosine. Chem Ind (London) 14:612-614.

Chang SS, Shen GH, Tang J, Jin QZ, Shi H, Carlin JT, and Ho CT. 1983. Isolation and identification of 2-pentenylfurans in the reversion flavor of soybean oil. J Amer Oil Chem Soc 60:553-557.

Chang SS, Smouse TH, Krishnamurthy RG, Mookherjee BD, and Reddy RB. 1966. Isolation and identification of 2-pentyl-furan as contributing to the reversion flavour of soyabean oil. Chem Ind (London) 1926-1927.

Corey EJ and Taylor WC. 1964. A study of the peroxidation of organic compounds by externally generated singlet oxygen molecules. J Amer Chem Soc, 86:3881.

Corwin H, Shellhammer TH. 2000. Combined Carbon Dioxide and High Pressure Inactivation of Pectin Methylesterase, Polyphenol Oxidase, *Lactobacillus plantarum*, and *Escherichia coli*. Paper # 62-3. Annual Meeting of the Institute of Food Technologists, Dallas, Texas, June 10 – 14.

Darmanyan AP and Jenks WS. 1998. Charge-transfer quenching of singlet oxygen $O_2(^1\Delta g)$ by amines and aromatic hydrocarbons. J Phys Chem 102:7420-7426.

Dimick PS. 1982. Photochemical effects on flavor and nutrients of fluid milk. Can Inst Sci Tech J 15:247-256.

Dimick PS and Kilara A. 1983. Photooxidative changes in milk and amino acids. Kieler Milchwirtsschaftliche Forschungsberichte 35:289-299.

Doleiden FH, Farenholtz SR, Lamola AA, and Trozzolo AM. 1974. Reactivity of cholesterol and some fatty acids toward singlet oxygen. Photochem Photobiol 20:519-521.

Donsì G, Ferrari G, Mateo MD. 1996. High-pressure stabilization of orange juice: Evaluation of the effects of process conditions. Ital J Food Sci 2:99-106.

Dunkley WL, Franklin JD, and Pangborn RM. 1962. Effects fo fluorescent light on flavor, ascorbic acid and riboflavin in milk. Food Tech 16:112-118.

Eagerman BA, Rouse AH. 1976. Heat inactivation temperature-time relationships for pectinesterase inactivation in citrus juices. J Food Sci 41:1396-1397.

Ensor, D.R. 1989. Flavor Chemistry in the Food Industry. In, Flavor Chemistry of Lipid Foods, Min, D.B. and Smouse, T.H., editors. pp. 1-12. The American Oil Chemists' Society, Champaign, Illinois.

Farnworth ER, Lagace M, Couture R, Yaylayan V, Stewart B. 2001. Thermal processing, storage conditions, and the composition and physical properties of orange juice. Food Res Int 34:25-30.

Foote CS. 1976. Photosensitized oxidation and singlet oxygen: consequences in biological systems. In: Pryor, WA, editor. Free Radicals in Biology. Vol. 2. New York: Academic Press. p 85.

Foote CS, Chang YC, and Denny RW. 1970. Chemistry of singlet oxygen. X. Carotenoid quenching parallels biological protection. J Am Chem Soc 92:5216-5219.

Foote CS and Denny RW. 1968. Chemistry of singlet oxygen quenching by β-carotene. J Am Chem Soc 90:6232-6238.

Foote CS and Wexler S. 1964. Olefin oxidations with excited singlet molecular oxygen. J Amer Chem Soc, 86:3879.

Forss DA. 1979. Mechanism of formation of aroma compounds in milk and milk products. J Dairy Res 46:691-694.

Frankel EN. 1985. Chemistry of autoxidation: Mechanism, products and flavor significance. In: Min DB and Smouse TH, editors. Flavor Chemistry of Fats and Oils. American Oil Chemists' Society. p 1-34.

Frankel EN, Neff WE, and Bessler TR. 1979. Analysis of autoxidized fats by gas chromatography-mass spectrometry: V. Photosensitized oxidation. Lipids 14:961-967.

Frankel EN, Neff WE, and Selke E. 1981. Analysis of autoxidized fates by gas chromatography-mass spectrometry: VII. Voalatile thermal decomposition products of pure hydroperoxides from autoxidized and photosensitized oxidized methyl oleate, linoleate and linolenate. Lipids 16:279-285.

Fukuzawa K. 2000. Singlet oxygen scavenging in phospholipid membranes. In: Packer L and Sies H, editors: Methods in Enzymology Volume 319. New York: Academic Press. 101-110.

Gardner, H.W. 1989. How the Lipoxygenase Pathway Affects the Organoleptic Properties of Fresh Fruit and Vegetables. In, Flavor Chemistry of Lipid Foods, Min, D.B. and Smouse, T.H., editors. pp. 98-112. The American Oil Chemists' Society, Champaign, Illinois.

Girotti AW. 1998. Lipid hydroperoxide generation, turnover, and effector action in biological systems. J Lipid Res 39:1529-1542.

Girotti AW and Korytowski W. 2000. Cholesterol as a singlet oxygen detector in biological systems. In: Packer L and Sies H, editors: Methods in Enzymology Volume 319. New York: Academic Press. p 85-100.

Golbitz P, editor. 2000. 2001 Soya & oilseed bluebook. Soyatech, Inc. Bay Harbor, ME. p 341-342.

Gollnick K. 1968. Type II photooxygenation reactions in solution. In: Noyes WA, Jr., Hammond GS and Pitts JN, Jr., editors. Advances in Photochemistry. Vol. 6. New York: Jonh Wiley & Sons. p 1-122.

Goodner JK, Braddock RJ, Parish ME. 1998. Inactivation of pectinesterase in orange and grapefruit juices by high pressure. J Food Sci 45(5):1997-2000.

Gunston FD. 1986. Chemical Properties. In: Gunston FD, Harwood JL, and Padley FB: The Lipid Handbook. New York, Chapman and Hall. p 449-484.

Harper WJ and Brown DJ. 1964. Light-induced degradation of cysteine. J Dairy Sci 47:548-552.

He JA, Hu YZ, and Jiang LJ. 1997. Photodynamic action of phycobiliproteins: *in situ* generation of reactive oxygen species. Biochimica et Biophysica Acta 1320:165-174.

He YY, An JY, and Jiang LJ. 1998. EPR and spectrophotometric studies on free radicals (O_2*, Cysa-HB*) and singlet oxygen ($^1O_2$) generated by irradiation of cysteamine substituted hypocrellin B. International Journal of Radiation Bioloogy 74:647-654.

Herreid EO and Ruskin B. 1952. Ascorbic acid and riboflavin loss and flavor development in milk exposed to the sun in amber, clear, paper and ruby bottles. J Dairy Sci 35:772-778.

Herzberg G. 1934. Photography of infrared solar spectrum to wavelength 12,900A°. Nature, London, 133, 759.

Ho CT, Smagula MS, and Chang SS. 1978. The synthesis of 2-(1-pentenyl) furan and its relationship to the reversion flavor of soybean oil. J Amer Oil Chem Soc 55:233-237.

Hoskin JC. 1979. Sensory analysis and riboflavin analysis of milk held in light-exposed one-gallon containers. M.S. Thesis, The Pennsylvania State University, University Park.

Hsieh, R.J. 1994. Contribution of Lipoxygenase Pathway to Food Flavors. In, Lipids in Food Flavors, Ho, C.T. and Hartman, T.G., editors. pp. 30-48. American Chemical Society, Washington DC.

Jia M, Zhang QH, Min DB. 1998. Optimization of solid-phase microextraction analysis for headspace flavor compounds of orange juice. J Agric Food Chem 46:2744-2747.

Jordan, MJ, Tillman, TN, Mucci, B, and Laencina, J. 2001. Using HS-SPME to determine the effects of reducing insoluble solids on aromatic composition of orange juice. Lebensm.-Wiss. u.-Technol. 34:244-250.

Jung MY, Kim SK, and Kim SY. 1995. Riboflavin-sensitized photoxidation of ascorbic acid: kinetics and amino acid effects. Food Chem 53:397-403.

Jung MY, Lee HO, and Min DB. 1998. Singlet oxygen and ascorbic acid effects on dimethyl disulfide and off-flavor in skim milk exposed to light. J Food Sci 63:408-412.

Jung YJ, Lee E, and Min DB. 1991. α-, γ-, δ-tocopherol effects on chlorophyll photosensitized oxidation of soybean oil. J Food Sci 45:183-187.

Kaanane A, Kane D, Labuza TP. 1988. Time and temperature effect on stability of Moroccan processed orange juice during storage. J Food Sci 53(5):1470-1473, 1489.

Klavons JA, Bennett RD, Vannier SH. 1994. Physical/chemical nature of pectin associated with commercial orange juice cloud. J. Food Sci 59:399-401.

Kanofsky JR. 2000. Assay for singlet-oxygen generation by peroxidases using 1270-nm chemiluminescence. In: Packer L and Sies H, editors: Methods in Enzymology Volume 319. New York: Academic Press. 59-67.

Kanofsky JR and Axelrod B. 1986. Singlet oxygen production by soybean lipoxygenase isozymes. J Biological Chem 261:1099-1104.

Ke PJ and Ackman RG. 1973. Bunsen coefficient for oxygen in marine oils at various temperatures determined by exponential dilution method with a polarographic oxygen electrode. J Amer Oil Chem Soc 50:429-435.

Kepka A and Grossweiner LI. 1972. Photodynamic oxidation of iodide ion and aromatic amino acids by eosine. Photochem Photobiol 14:621-639.

King JM. 1996. Riboflavin photosensitized singlet oxygen oxidation of vitamin D. Ph.D. Dissertation. The Ohio State University, Columbus.

King JM and Min DB. 1998. Riboflavin photosensitized singlet oxygen oxidation of vitamin D. J Food Sci 63:31-34.

Klavons JA, Bennett RD, Vannier SH. 1994. Physical/chemical nature of pectin associated with commercial orange juice cloud. J. Food Sci 59:399-401.

Kobayashi M and Sakamoto Y. 1999. Singlet oxygen quenching ability of astaxanthan esters from the green alga *Haematococcus pluvialis*. Biotech Letters 21:265-269.

Kochevar IE and Redmond RW. 2000. Photosensitized production of singlet oxygen. In: Packer L and Sies H, editors: Methods in Enzymology Volume 319. New York: Academic Press. 20-28.

Korycka-Dahl MB and Richardson T. 1978. Activated oxygen species and oxidation of food constituents. Crit Rev Food Sci Nutr 10:209-240.

Koseoglu SS and Lusas EW. 1990. Chapter 8: Hydrogenation of canola oil. In Shahidi F, editor. Canola and Rapeseed: Production, Chemistry, Nutrition and Processing Technology. Van Nostrand Reinhold Company Inc. New York pg 123-148.

Krinsky NI. 1977. Singlet oxygen in biological systems. Trends Biochem Sci 2:35-38.

Labuza TP. 1971. Kinetics of lipid oxidation in foods. CRC Crit Rev Food Sci Nutr 2:355-405.

Lea CH. 1952. Methods for determining peroxide in lipids. J Sci Food Agric 3:586-594.

Lee HS and Coates GA. 1999. Vitamin C in frozen, fresh squeezed, unthermally processed, polyethylene-bottled orange juice: a storage study. Food Chem 65:165-168.

Lee SH and Min DB. 1990. Effects, quenching mechanisms and kinetics of carotenoids in chlorophyll-sensitized photooxidation of soybean oil. J Agric Food Chem 38:1630-1634.

Li TL, King JM, and Min DB. 2000. Quenching mechanisms and kinetics of carotenoids in riboflavin photosensitized singlet oxygen oxidation of vitamin D_2. J Food Biochem 24: 477-492.

Li TL and Min DB. 1998. Stability and photochemistry of vitamin D_2 in model systems. J Fd Sci 63:413-417.

Lindsay, R.C. 1996. Flavors. In, Food Chemistry, 3rd edition, Fennema, O.R., editor. pp. 723-765. Marcel Dekker, Inc. New York.

Lledias F and Hansberg W. 2000. Catalase modification as a marker for singlet oxygen. In: Packer L and Sies H, editors: Methods in Enzymology Volume 319. New York: Academic Press. 110-119.

Macpherson AN, Tefler A, Barber J, and Truscott TG. 1993. Direct detection of singlet oxygen from isolated Photosystem II reaction centers. Biochimica et Biophysica Acta 1143:301-309.

Maniere FY and Dimick PS. 1976. Effect of fluorescent light on repartition of riboflavin content in homogenized milk. J Dairy Sci 59:2019-2022.

Martin, AB, Acree, TE, Hotchkiss, JH, Nagy S. 1992. J Agric Food Chem. 40:650-654.

Meyssami B, Balaban MO, Teixeira AA. 1992. Prediction of pH in model systems pressurized with carbon dioxide. Biotechnol Prog 8(2):149-154.

Michaeli A and Feitelson J. 1994. Reactivity of singlet oxygen toward amino acids and peptides. Photochem Photobiol 59:284-289.

Michaeli A and Feitelson J. 1995. Reactivity of singlet oxygen toward large peptides. Photochem Photobiol 61:255-260.

Michaeli A and Feitelson J. 1997. Reactivity of singlet oxygen toward proteins: the effect of structure in basic pancreatic trypsin inhibitor and in ribonuclease A. Photochem Photobiol 65:309-315.

Min DB. 2000. Recent developments in lipid oxidation and antioxidants. Canadian section of the American Oil Chemists' Society Meeting. November, 2000. Quebec, Ontario.

Min DB and Lee HO. 1996. Chemistry of lipid oxidation. In: McDonald RE and Min DB, editors. Food Lipids and Health. New York: Marcel Dekker, Inc. p 241-268.

Min DB and Lee EC. 1988. In: Charalambous G, editor. Frontiers of Flavor. Amsterdam: Elsevier. p 473-498.

Min DB and Lee HO. 1999. Chemistry of lipid oxidation. In: Teranishi R, Wick EL and Hornstein I, editors. Flavor Chemistry: Thirty Years of Progress. New York: Kluwer Academic/Plenum Publishers. p 175-187.

Min DB, Lee SH and Lee EC. 1989. Singlet oxygen oxidation of vegetable oils. In: Min DB and Smouse TH, editors: Flavor Chemistry of Lipid Foods. American Oil Chemists' Society, Champaign, IL. p 57-97.

Miskoski S and Garcia NA. 1993. Influence of the peptide bond on the singlet molecular oxygen-initiated ($O_2[^1\Delta g]$) photooxidation of histidine and methionine dipeptides. A kinetic study. Photochem Photobiol 57:447-452.

Moshonas MG, Shaw PE. 2000. Changes in volatile flavor constituents in thermally processed orange juice during storage. J Food Quality 23:61-71.

Mulliken RS. 1928. Interpretation of the atmospheric oxygen bands: electronic levels of the oxygen molecule. Nature, London. 122, 505.

Neff WE and Frankel EN. 1980. Quantitative analysis of hydroxystearate isomers from hydroperoxides by high pressure liquid chromatography of autoxidized and photosensitized-oxidized fatty esters. Lipids 15:587-590.

Nienaber U, Shellhammer TH. 2001. High-pressure processing of orange juice: Kinetics of pectinmethylesterase inactivation. J. Food Sci 66(2):328-331.

Nonell S and Braslavsky SE. 2000. Time-resolved singlet oxygen detection. In: Packer L and Sies H, editors: Methods in Enzymology Volume 319. New York: Academic Press. 37-49.

Ogawa H, Fukuhisa K, Kubo Y, Fukumoto H. 1990. Pressure inactivation of yeasts, molds, and pectinesterase in satsuma mandarin juice: effects of juice concentration, pH, and organic acids, and comparison with heat sanitation. Agric Biol Chem 54(5):1219-1225.

Parkes GD. 1967. Oxygen. In: Parker GD, editor. Mellor's modern inorganic chemistry. New York: John Wiley and Sons, Inc. P 331-355.

Patton S. 1954. The mechanism of sunlight flavor formation in milk with special reference to methionine and riboflavin. J Dairy Sci 37:446-452.

Patton S and Josephson DV. 1953. Origin of sunlight flavor in milk. Science 118:211.

Pecci L, Costa M, Antonucci A, Montefoschi G, and Cavallini D. 2000. Methylene Blue photosensitized oxidation of cysteine sulfinic acid and other sulfinates: The involvement of singlet oxygen and the azide paradox. Biochemical and Biophysical Research Communications 270: 782-786.

Pokorny, J. 1989. Flavor Chemistry of Deep Fat Frying in Oil. In, Flavor Chemistry of Lipid Foods, Min, D.B. and Smouse, T.H., editors. pp. 113-155. The American Oil Chemists' Society, Champaign, Illinois.

Priestley J. 1775. Experiments and Observation on Different Kinds of Air. London. (Experiments, Alembic Club Reprints No. 7, Edinburgh, 1775).

Rawls HR and VanSanten PJ. 1970. A possible role for singlet oxidation in the initiation of fatty acid autoxidation. J Amer Oil Chem Soc 47:121-125.

Redd JB, Hendrix CM Jr, Hendrix DL. 1986. Quality control manual for citrus processing plants. Intercit, Inc., Safety Harbor, FL.

Reynolds, TM. 1963. Chemistry of non-enzymatic browning. In Advances in Food Research, Vol. 12, C.O. Chichester, editors E.M. Mark and G.F. Stewart. Academic Press, London.

Rinken SA and Watherson JJ. 1993. Vitamin D stability in milk. J Food Sci. 58:552-556,566.

Rombouts FM, Versteeg C, Karman AH, Pilnik W. 1982. Pectinesterase in component parts of citrus fruits related to problems of cloud loss and gelation in citrus products. In: Dupuy P, editor. Use of enzymes in food technology. Paris: Technique et Documentaiton Lavoisier. p 483-487

Rouse AH, Atkins CD. 1955. Pectinesterase and pectin in commercial orange juice as determined by methods used at the Citrus Experiment Station. Bulletin of the University of Florida Agricultural Experiment Station, Lake Alfred, FL 570:1-19.

Sadler, GD, Parish, ME, Wicker L. 1992. Microbial, enzymatic, and chemical changes during storage of fresh and processed orange juice. J Food Sci 57:1187-1191.

Samuelson EG. 1962. Experiments on sunlight flavor in S-35 labeled milk. Milchwissenschaft 17:401-405.

Satter A and deMan JM. 1975. Photoxidation of milk and milk products. CRC Crit Rev Food Sci and Nutr 7:13-37.

Schneider T, Gugliotti M, Politi MJ, and Baptista MS. 2000. Quantitative determination of singlet oxygen by laser deflection calorimetry. Analytical Letters 33:297-305.

Sharman WM, Allen CM, and van Lier JE. 2000. Role of activated oxygen species in photodynamic therapy. In: Packer L and Sies H, editors: Methods in Enzymology Volume 319. New York: Academic Press.

Shaw PE, Buslig BS, Moshonas MG. 1993. Classification of commercial orange juice types by pattern recognition involving volatile constituents quantified by gas chromatography. J Agric Food Chem 41:809-813.

Smagula MS, Ho CT, and Chang SS. 1978. The synthesis of 2-(2-pentenyl) furans and their relationship to the reversion flavor of soybean oil. J Amer Oil Chem Soc 56:516-519.

Smouse TH and Chang SS. 1967. A systematic characterization of the reversion flavor of soybean oil. J Amer Oil Chem Soc 44: 509-514.

Snir R, Koehler PE, Sims KA, Wicker L. 1996. Total and thermostable pectinesterase in citrus juice. J. Food Sci 61(2):379-382.

Song YZ, An J, and Jiang L. 1999. ESR evidence of the photogeneration of free radicals (GDHB*, O_2*) and singlet oxygen ($^1O_2$) by 15-deacetyl-13-glycine-substituted hypocrellin B. Biochimica et Biophysica Acta. 1472:307-313.

Spikes JD and Bommer JC. 1991. Chlorophyll and related pigments as photosensitizers in biology and medicine. In Chlorophylls, Scheer H. 1181-1204.

Steffan A, Pawliszyn J. 1996. Analysis of flavor volatiles using headspace solid-phase microextraction. J Agric Food Chem 44:2187-2192.

Stratton SP and Liebler DC. 1997. Determination of singlet oxygen-specific versus radical-initiated lipid peroxidaition in photosensitized oxidation of lipid bilayers: Effect of beta-carotene and alpha-tocopherol. Biochem 36:12911-12920.

Swern D. 1979. Bailey's Industrial Oil and Fat Products, vol. 1. New York: Wiley. 177-233.

Telfer A, Bishop SM, Phillips D, and Barber J. 1994. Isolated photosynthetic reaction center of photosystem II as a sensitizer for the formation of singlet oxygen. J Biol Chem 269:13244-13253.

Truong T, Shellhammer TH. 2001. High-pressure inactivation of pectinmethylesterase enhanced by carbon dioxide. Annual Meeting of the Institute of Food Technologists, New Orleans, LA, June 25.

United States Public Health Services/Food and Drug Administration. 1993. Grade "A" Pasteurized Milk Ordinance. U.S. Department of Health and Human Services, Publication No. 229. p 259.

Usuki R, Endo Y, and Kaneda T. 1984. Prooxidant activities of chlorophylls and pheophytins on the photooxidation of edible oils. Agric Biol Chem 48:991-994.

Van den Broeck I, Ludikhuyze LR, Van Loey AM, Weemanes CA, Hendrickx ME. 1999. Thermal and combined pressure-temperature inactivation of orange pectinesterase: Influence of pH and additives. J Agric Food Chem 47(7):2950-2958.

Versteeg C, Rombouts FM, Spansen CH, Pilnik, W. 1980. Thermostability and orange juice cloud destabilizing properties of multiple pectinesterases from orange. J Food Sci 45:969-971, 998.

Wercinski, S.A.S. and Pawliszyn J. 1999. Solid Phase Microextraction Theory. In, Solid Phase Microextraction. Wercinski, S.A.S., editor. pp. 1-26. Marcel Dekker, Inc. New York.

Whang K and Peng IC. 1988. Electron paramagnetic resonance studies of the effectiveness of myoglobin and its derivatives as photosensitizers in singlet oxygen generation. J Food Sci 53:1863-1865.

White CH and Bulthaus M. 1982. Light activated flavor in milk. J Dairy Sci 65:489-494.

Wicker L, Temelli F. 1988. Heat Inactivation of pectinesterase in orange juice pulp. J. Food Sci 53(53):162-164.

Wishner LA. 1964. Light induced oxidations in milk. J Dairy Sci 47:216-221.

Yang WTS. 1994. Effects of synthetic food colorants on singlet oxygen oxidation of foods. Ph.D. Dissertation. The Ohio State University, Columbus.

Yang WTS and Min DB. 1994. Chemistry of singlet oxygen oxidation of foods. In: Ho CT and Hartmand TG, editors. Lipids in Food Flavors. Washington DC: American Chemical Society. p 15-29.

Yeom HW, Streaker CB, Zhang QH, Min DB. 2001. Effects of pulsed electric fields on the quality of orange juice and comparison with heat pasteurization. J Agric Food Chem. 48:4597-4605.